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TITLE: Psycho-Endocrine-Immune Profile: Implications for Quality of Life In Breast Cancer Patients

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13. ABSTRACT *(Maximum 200 Words)*

Breast biopsy is an emotional experience that may impair anti-tumor immune responses. This study determined a woman's psychological and immune response pre (T1 and T2) and post (T3 and T4) breast biopsy. Overall, stress, anxiety, and mood disturbance were heightened pre-biopsy. Post-biopsy, stress, anxiety, and mood disturbance diminished but remained increased relative to non-biopsied control women. Natural killer cell activity (NKCA) was depressed pre- and post- biopsy compared to non-biopsied control women. Women with cancer had greater stress and lower NKCA post-biopsy compared to women with benign results. IL-6 production by peripheral blood mononuclear cells of biopsy patients was increased both pre- and post-biopsy compared to control women. Conversely, IFNγ and IL-2 were depressed at T1-T4, while IL-4 and IL-10 were increased at T2 and T3 and normalized by T4 relative to control women. Thus, breast biopsy produced stress, anxiety, and mood disturbance, which was diminished post-biopsy. Breast biopsy was accompanied by depressed NKCA and altered cytokine production that persisted well beyond the biopsy experience and were more marked in women diagnosed with breast cancer. In conclusion, psychological and immune dysregulation begin early in a woman's encounter with breast cancer and may influence cancer control.

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(4) INTRODUCTION

In American women, breast cancer is the most common cancer and the second leading cause of cancer death (1). Living through the experience of cancer diagnosis and treatment is emotionally challenging and this emotional experience often begins with the realization that a breast biopsy is deemed necessary. Almost a million women undergo breast biopsy annually (2). Biopsy of the breast is often a critical life event and the concomitant uncertainty and fear of a cancer diagnosis is stressful. Although environmental stress is known to impair the immune response to tumor cells, stress remains poorly understood. Yet stress is a modifiable risk factor that can impact anti-tumor immune responses (3). The stress of an impending cancer diagnosis is a human paradigm in which coherent links between a woman's psychological and immunological states can be formed. The purpose of this ongoing study is to measure psychological stress. stress hormones, and the immune response to tumor cells in women before and after breast biopsy. The design of this study will allow the exploration of the biological links between stress and immune function at an early point in a woman's encounter with cancer.

Recent advances in the disciplines of psychoneuroimmunology and neuro-immunomodulation have provided intriguing evidence that psychological stress can lead to profound changes in immune function, especially Natural Killer (NK) cell cytotoxicity (4-7). NK cells are lymphocytes that mediate natural resistance against tumor cells and viruses and are particularly important in the control of tumor metastasis. Hence, NK cells are pivotal to the control of cancer and disease outcome (8, 9). Patients with a variety of solid tumors (e.g., breast, cervix, endometrium, ovary, and lung) exhibit reduced NK cell activity (NKCA) (8). Indeed, lower NKCA has been reported to predict disease recurrence in breast cancer (8, 9). A significant health-related implication of research in psychoneuroimmunology is that stress-induced immunomodulation may influence the progression of malignancy. The seminal study of Spiegel et al. demonstrated that a structured behavioral intervention program that reduced stress in women with advanced metastatic breast cancer, resulted in an overall increase in cancer survival (10, 11). These results are intriguing, yet no immune parameters were monitored and women entered the study at an advanced stage of disease. More recently, Andersen et al., studied the stress-immune link in women with breast cancer at a point in time after surgical removal of their tumor, but prior to initiation of adjuvant chemo- or radiotherapy. Their results indicated that women with breast cancer who report high levels of perceived stress have low levels of NKCA against tumor cells and low responsiveness of their NK cells to interferon (IFN) (12). The results of these key studies are highly intriguing and clearly support the need for research studies designed to unravel the underlying biologic mechanisms of stress-induced immunsuppression and cancer progression.

The mechanisms underlying stress-induced impairment in NKCA are not well understood. It is possible that stress may directly alter NKCA or alternatively it may indirectly alter NKCA by changing Th1/Th2 cytokines. Cytokines are important regulatory factors that modulate NK cell cytotoxicity (14-16). It is possible that stress exerts its effects on NKCA by altering cytokine secretion. Although little research has been conducted in humans, stress is hypothesized to alter the Th1/Th2 pattern of cytokine secretion so that there is a reduced production of Th1 cytokines. Th1 cytokines, which include IFNγ and IL-2, are important activators of NKCA against tumor cells. The production of these cytokines is reduced by adrenal glucocorticoids (cortisol) and augmented by DHEA/DHEAS (13, 17-19). Since stress activates the hypothalamic-pituitary-adrenal (HPA) axis resulting in an increase in adrenal cortisol and a decrease in adrenal DHEA and DHEAS , (18, 19) it is the purpose of this study to evaluate whether stress-induced changes in steroid hormone secretion play a role in the depressed NKCA observed in women stressed by impending cancer diagnosis and therapy. It is likely that an altered steroid hormone profile modulates the cytokine pattern, such that NKCA is reduced. Therefore, the purpose of the proposed study is to determine

the links among psychological stress, steroid hormones, and immune function (e.g., cytokine profile and NKCA). It is hypothesized that psychological stress due to potential cancer diagnosis and treatment induces a steroid hormone profile, (increased cortisol and decreased DHEAS) that mediates a change in the Th1/Th2 cytokine pattern. These changes in hormones and cytokines may directly and/or indirectly affect NKCA.

The technical objectives of this project are:

(1) Determine the psycho-endocrine and NK cell response of women to the experience of breast biopsy for cancer diagnosis.

(2) Identify the importance of Th1/Th2 cytokines in the psycho-endocrine-NK cell response of women undergoing breast biopsy for cancer diagnosis.

(3) Understand differences in the psycho-endocrine-immune profile of women with benign versus malignant breast biopsy findings.

(5) BODY

Methods - All women over 18 years of age, who seek consultation at the Breast Care Center of Loyola University's Cancer Center and who subsequently receive a breast biopsy (excluding fine needle aspirates) were eligible subjects. Exclusionary criteria include: pregnancy, prior or current history of cancer, recent history of major psychiatric disorder or concurrent major immune-based disease, and active substance abuse. Women were studied at the following four time points:

- T_1 - Initial consultation at the Breast Care Center
- T_2 - Day of biopsy, prior to the actual biopsy
- T_3 - At return clinic visit, 10-14 days after notification of biopsy results
- T_4 - 1-2 months after T_3.

The rationale for these time periods is that the first two are pre-biopsy and are likely to represent a period of stress; whereas, the latter two occur after notification. For women with negative findings T_3 and T_4 will likely reflect resolution of the stress of breast biopsy, while women with positive findings, will likely remain stressed. At each time period volunteers completed informed consent documents, had their blood drawn, and completed the psychological measures and a health history questionnaire. Clinical review of medical records compared outcomes between malignant and non-malignant goups of women.

Psychological Measures: Psychological stress was defined as an individual's response to perturbations from the environment (i.e., breast biopsy and possible cancer diagnosis). The psychological measures included a visual analogue scale for global stress and for biopsy-related stress, Cohen's Perceived Stressor Scale, Speilberger's State Trait Anxiety Inventory, and the Profile of Mood States.

Laboratory Measures: Serum was stored for the analysis of stress hormone levels (cortisol, DHEA/DHEAS) by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Peripheral blood mononuclear cells (PBMC) were derived immediately by Ficoll/Hypaque separation. These isolated PBMC's were assessed for NKCA against [^{51}Cr] labeled K562 tumor targets as described previously (20). Cytokine production was measured by stimulation of peripheral blood mononuclear cells in bulk culture. Culture supernatants were collected after 48 hrs and cytokines were measured using standard ELISA kits

(R & D Systems, Minneapolis, MN).

Statement of Work To Date.

Task 1. Determine the psycho-endocrine and NK cell response of women to the experience of breast biopsy for cancer diagnosis (months 1-36). Thirty-seven women have been enrolled in this study over the past year. The total number of women enrolled in this study to date in 110. Of the total women enrolled in this study, 12 women did not have a biopsy performed. This was often a clinical decision occurring on the day of biopsy prior to the actual procedure. Eight women withdrew from the study. Of the 90 women undergoing breast biopsy, 66 had benign biopsy results (80%), while 24 women were diagnosed with malignancy (27%). Eighty-six women were White; 10 African American; 4 Hispanic; 2 Asian; and 4 were Other/Unknown. The ages of subjects ranged from 18 to 85 years of age. The mean±SD age of all subjects was 53±14 years (benign 49± 14; malignant 62± 11). Not all women were able to complete all four data collection periods; hence, the subject number varies per data collection time period. The data that is reported herein is reported for the total number of women enrolled to date and is reported as means and standard errors of the mean. At this time point in the conduct of this project, no statistical analysis has been performed but we are nearing this point in the study. The psycho-endocrine stress profile and NK cell activity of each subject pre and post breast biopsy was measured. Each individual was administered psychological instruments, had blood drawn and peripheral blood mononuclear cells isolated for measure NK cell activity and plasma adrenocortical stress hormones (cortisol, DHEAS). Not all endocrine and cytokine assays have been completed on frozen serum samples. Relevant demographic, medical history, and disease characteristics from medical records were abstracted and contact with subjects was maintained per telephone to ensure subject retention, and quality control of laboratory and psychological data collection has been accomplished. These data are presented below and illustrated in Figures 1-14.

Task 2. Identify the importance of the Th1/Th2 cytokine profile to the psycho-endocrine-NK cell response of women undergoing breast biopsy for cancer diagnosis (month 1-36). For each of the blood samples from the above subject, cytokine production was measured pre and post biopsy. These data are presented below and illustrated in Figures 10-14.

Task 3. Understand differences in the psycho-endocrine-immune profile of women with benign versus malignant breast biopsy findings (months 24-36). Our malignant enrollment has doubled during this past year and now beginning comparisons can be made. As appropriate, beginning comparisons are presented between benign and malignant groups and are depicted in Figures 1-14. Cytokine variables are presented for the total group and the stratification of this data into benign and malignant groups.

Task 4. Analysis and Manuscript Preparation (months 24-36) will be accomplished during the upcoming year. One article is in press and one related chapter has been published. Three abstracts have been prepared and presented at scientific conferences.

Summary of Results

Figures 1 and 2 illustrate a comparison of the results for women in the benign and malignant groups on the two visual analogue scales used in this study. Global stress (Figure 1) indicated overall stress in a woman's life at the moment, while biopsy stress (Figure 2) indicated the level of stress a woman was experiencing because of her biopsy. Hence, global stress was more comprehensive and biopsy stress was more specific to the biopsy experience. Both were measured using 10-cm visual analogue scales. The results indicate that the two pre biopsy time points were times of stress for all women; however, both global stress and biopsy stress levels decreased from pre to post biopsy in the benign group. In contrast, stress for the

malignant group continued at higher levels than that reported by the women in the benign group. This is most marked in the measure of biopsy stress (Figure 2).

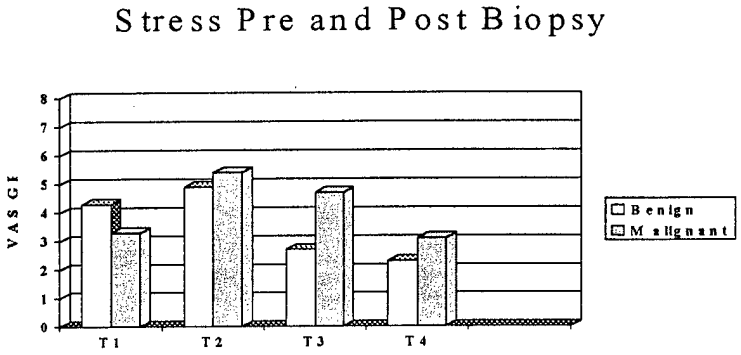

Figure 1 - Stress pre (T1 and T2) and post (T3 and T4) breast biopsy was measured by use of a 10 cm visual analogue scale that determined global stress at the moment (VAS GBL). Bars represent the mean value for benign and malignant groups. Standard errors of the mean ranged from 0.3-0.4 in the benign group and 0.6-0.9 in the malignant group. For the benign group N=43, 51, 48, and 45 for each respective time point; whereas for the malignant group N=12, 20, 18, and 15.

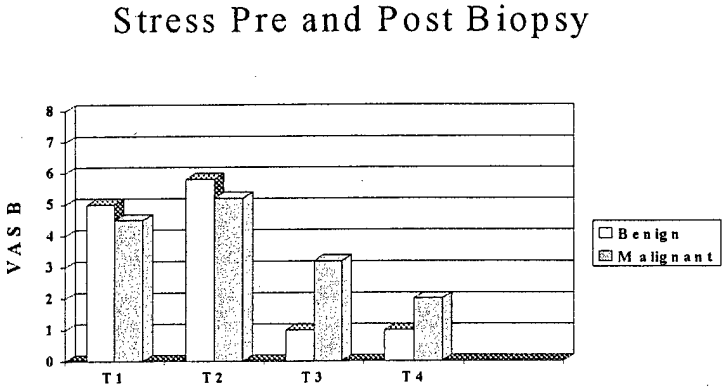

Figure 2 - Stress pre (T1 and T2) and post (T3 and T4) breast biopsy was measured by use of a 10 cm visual analogue scale that determined stress related to the biopsy experience (VAS Bx). Bars represent the mean value for benign and malignant groups. Standard errors of the mean ranged from 0.2-0.4 in the benign group and 0.5-0.9 in the malignant group. For the benign group N=54, 60, 37, and 41 for each respective time point; whereas for the malignant group N=15, 21, 13, and 14.

Stress Pre and Post Breast Biopsy

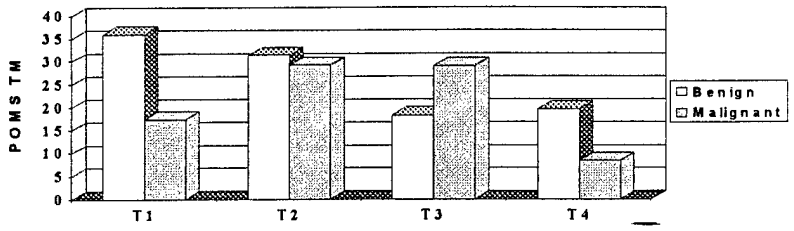

Figure 3 – Total mood disturbance (TMD) was measured using the Profile of Mood States (POMS). Women were assessed pre and post breast biopsy. Bars represent the mean value for benign and malignant groups. Standard errors of the mean ranged from 3.8-5.3 in the benign group and 7.7-9.8 in the malignant group. For the benign group N=51, 61, 51, and 53; whereas for the malignant group N=15, 18, 19, 16 for each time point.

Figure 3 shows the levels of total mood disturbance (TMD), as measured by the Profile of Mood States (POMS), for benign and malignant groups. TMD was elevated in both groups pre-biopsy (i.e., T1 and T2). Post-biopsy (T3) mood disturbance markedly decreased in the benign group but remained elevated in the malignant group. The greater mood disturbance in the benign group compared to the malignant group is unclear.

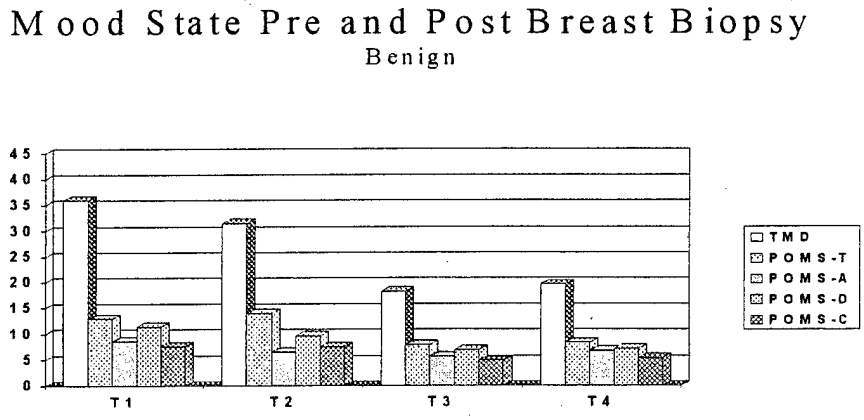

Figure 4 – The mood state is depicted pre (T1 and T2) and post (T3 and T4) breast biopsy for women in the benign group. Mood state was measured using the Profile of Mood States (POMS) and the total mood disturbance (TMD) is depicted along with the levels for subscales indicating tension (POMS-T), anger (POMS-A), depression (POMS-D), and confusion (POMS-C). Bars represent the mean for each mood state. Standard errors of the mean ranged from 0.6-1.9. N ranged from 51-61 per group.

The POMS is composed of six subscales that measure Tension, Anger, Depression, Confusion, Fatigue, and Vigor. Figures 4 and 5 show changes over time in four of the mood state subscales (Note: Vigor and Fatigue are illustrated in Figures 6 and 7.) In the benign group (Figure 4), each of the mood state subscales decreased from pre to post biopsy. Hence, mood disturbance is characterized by higher levels of tension, anger, depression, and confusion in women diagnosed with breast cancer compared to women who are found to have benign biopsy results.

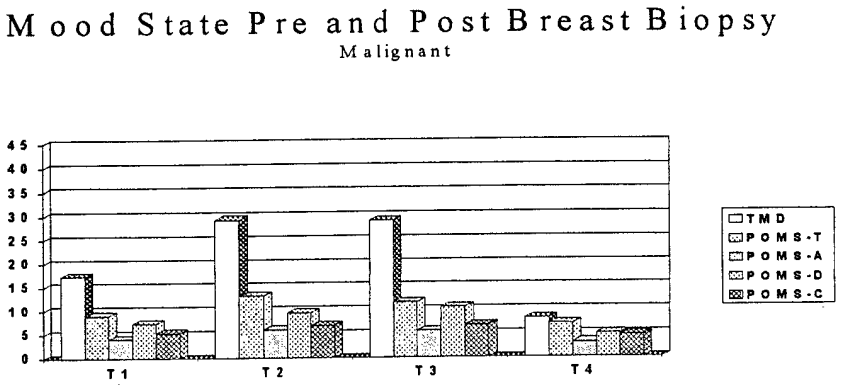

Figure 5 – The mood state is depicted pre (T1 and T2) and post (T3 and T4) breast biopsy for women in the malignant group. Mood state was measured using the Profile of Mood States (POMS) and the total mood disturbance (TMD) is depicted along with the levels for subscales indicating tension (POMS-T), anger (POMS-A), depression (POMS-D), and confusion (POMS-C). Bars represent the mean for each mood state. Standard errors of the mean ranged from 0.8-2.7. N ranged from 15-19 for each of the bars.

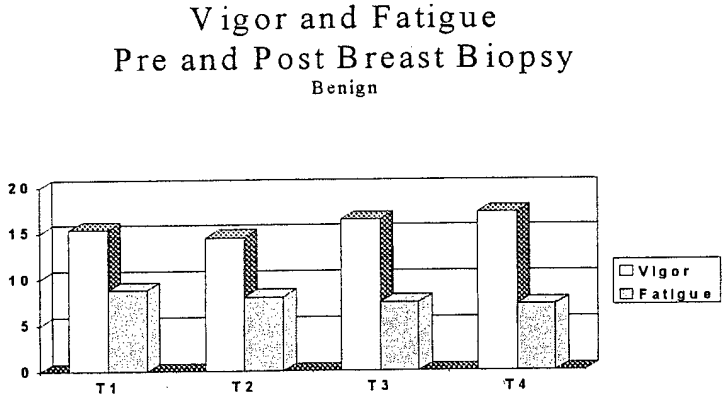

Figure 6 – Levels of vigor and fatigue are illustrated for women in the benign group at pre (T1 and T2) and post (T3 and T4) breast biopsy time periods. Vigor and fatigue were measured using the Profile of Mood States. Bars represent the mean values. Standard errors of the mean ranged from 0.7-0.9. N ranged from 51-61 per group.

Levels of fatigue and vigor do not change markedly from pre to post biopsy in either the benign or malignant groups, although vigor tends to increase and fatigue to decrease (Figures 6 and 7).
Anxiety, as measured by Speilberger's State Anxiety Inventory (STAI), shows decreases from pre to post biopsy in both benign and malignant groups; however, no dramatic differences between these groups are observed at any of the time points (Figure 8). As expected , trait anxiety remains stable from pre to post biopsy in the benign group. Data not shown. Trait anxiety was also similar for the malignant group.
Perceived stress was measured using Cohen's Perceived Stressor Scale, which assesses stress over the past month. Perceived stress ranged showed no change within groups over time or between groups at each time point. Data not shown. Because of the time period for this instrument (i.e., perceived stress over the past month), it is likely that this instrument is not sensitive to changes in stress level over shorter time periods like those experienced by women in this study.

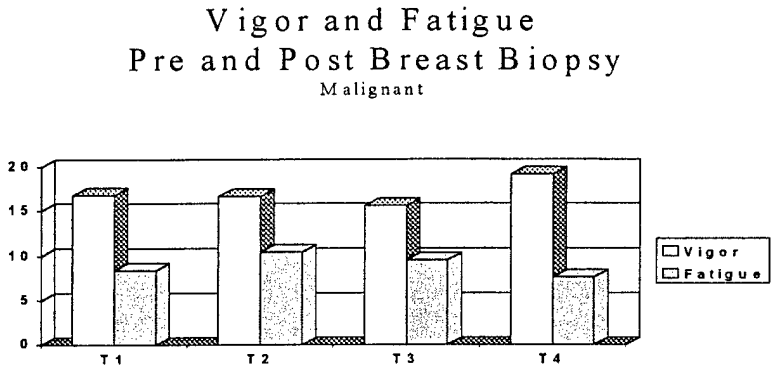

Figure 7 – Levels of vigor and fatigue are illustrated for women in the malignant group at pre (T1 and T2) and post (T3 and T4) breast biopsy time periods. Vigor and fatigue were measured using the Profile of Mood States. Bars represent the mean values. Standard error of the mean ranged from 1.6-2.1. N ranged from 15-19 for each of the bars.

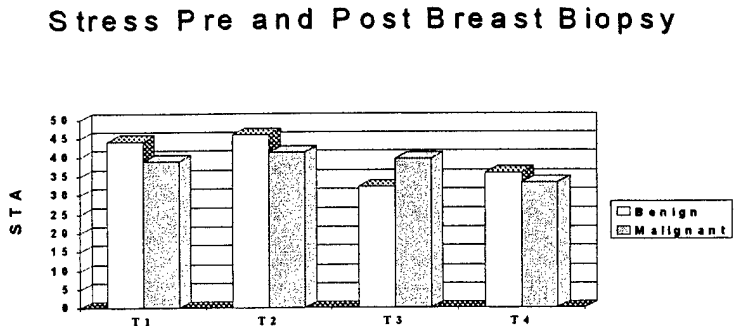

Figure 8 - Levels of state anxiety are illustrated for women in the benign and malignant groups. State anxiety was measured pre (T1 and T2) and post (T3 and T4) breast biopsy using Spielberger's State Anxiety Inventory (STA). Bars represent the mean values. Standard error of the mean ranged from 1.5-3.9. For the benign group, N=53, 62, 53, and 53 for each respective time; whereas, N=15, 22, 19, and 16 for the malignant group.

Psychological summary: The experience of breast biopsy produces psychological distress in women as indicated by increased levels of self-reported stress, anxiety, and mood disturbance. Altered mood is characterized by increased tension, depression, and confusion. Post biopsy, psychological perceptions of stress, tension, depression, and confusion remain elevated in women diagnosed with breast cancer. These data clearly demonstrate that the stress of breast cancer begins at the time of diagnosis, that it is present for all women pre-biopsy and that it decreases for women with benign results whereas it remains sustained for women with a malignant diagnosis.

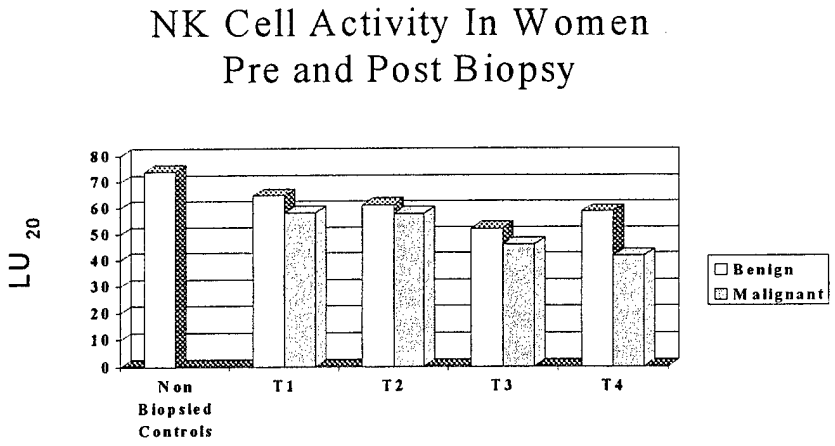

Figure 9 - Natural Killer (NK) cell activity, expressed as lytic units at 20%, is illustrated for non-biopsied control women and women in the benign and malignant groups. Peripheral blood was collected pre (T1 and T2) and post (T3 and T4) breast biopsy. NK cell activity was measured using K562 tumor cells as the target. Bars represent mean values. Standard errors of the mean ranged from 3.7-9.7. For the benign group, N=50, 61, 54, 51 for each respective time point; whereas, N=12, 22, 17, 16 for the malignant group. N=12 for the non-biopsied control group.

The NKCA (lytic units at 20%) for women pre and post breast biopsy is illustrated in Figure 9. In comparison to non-biopsied control women, NKCA is depressed in both benign and malignant groups for each time period. Although the amount of NKCA depression is similar within groups over time, the most marked depression in NKCA is noted in the malignant group at T4. At T4 NKCA is depressed compared to non-biopsied control women and compared to women in the benign group. Phenotypic analysis for circulating NK cells showed no differences between normal control subjects and biopsy patients. Data not shown.

The results of cytokine production are shown in Figures 10-14. IL-2 production showed no dramatic change, with possibly a slight reduction, for both the benign or the malignant groups T1 - T4 when compared to control non-biopsied women (Figure 10). IL-6 showed increased levels at all time points for both groups of women compared to the non-biopsied control women (Figure 11). IL-4 and IL-10 showed increased production throughout the biopsy experience and when compared to the non-biopsied control subjects, Figures 12 and 13. In contrast, IFNγ was decreased in its production at all time points for both the malignant and benign groups. This initial data set indicates that the experience of biopsy does produce alterations in the production of cytokines that are sustained beyond the biopsy period and beyond the period of psychological stress as reported by these women. No cytokines were detected in the serum of representative control or biopsy subjects. Data not shown.

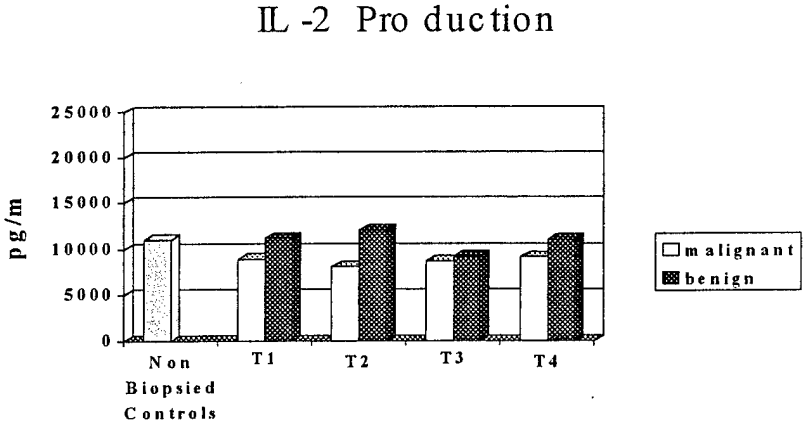

Figure 10 - Mononuclear cell production of IL-2 is depicted for non-biopsied control women and women pre (T1 and T2) and post (T1 and T2) breast biopsy. Peripheral blood mononuclear cells were activated with PMA/PHA and culture supernatants were collected at 48 hrs. IL-2 was determined by enzyme-linked immunoabsorbent assay. Bars represent the mean values. Standard error of the mean ranged from 2300-2700. N= 8 for controls and N= 27, 26, 26, 23 for the benign group and N= 7, 11, 7, 8 for the malignant group.

IL-6 Production

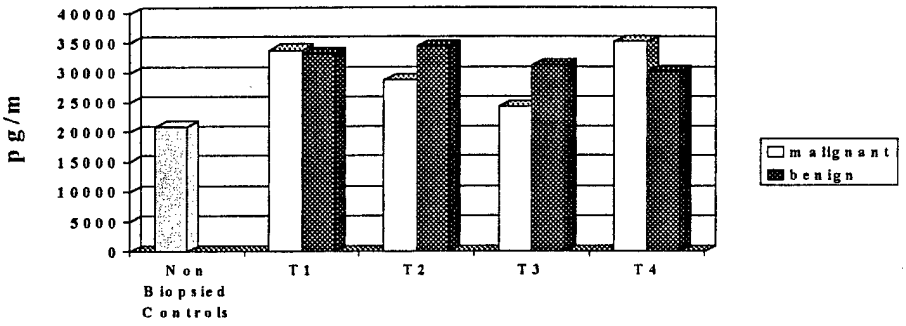

Figure 11 - Mononuclear cell production of IL-6 is depicted for non-biopsied control women and women pre (T1 and T2) and post (T3 and T4) breast biopsy. Peripheral blood mononuclear cells were activated by PMA/PHA and culture supernatants were collected at 48 hrs. IL-6 was determined by enzyme-linked immunoabsorbent assay. Bars represent the mean values. Standard error of the mean ranged from 2500-7000. N = 7 for controls and N= 31, 31, 34, 29 for the benign group and N = 7, 15, 10, 10 for the malignant group.

IL-4 Production

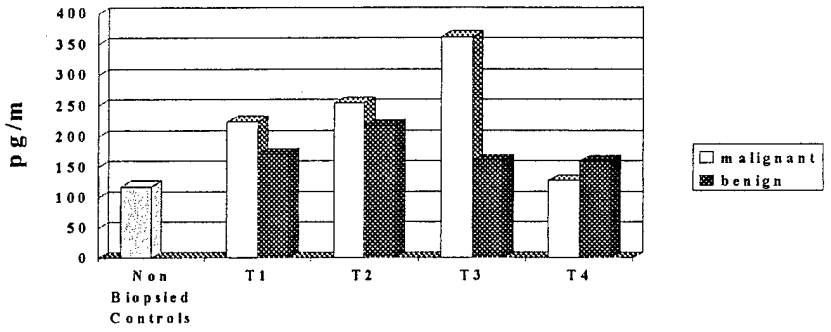

Figure 12 - Mononuclear cell production of IL-4 is depicted for non-biopsied control women and women pre (T1 and T2) and post (T3 and T4) breast biopsy. Peripheral blood mononuclear cells were activated by PMA/PHA and culture supernatants were collected at 48 hrs. IL-4 was determined by enzyme-linked immunoabsorbent assay. Bars represent the mean values. Standard error of the mean ranged from 26-156. N= 7 for controls and N = 34, 39, 36, 31 for the benign group and N = 11, 16, 11, 10 for the malignant group.

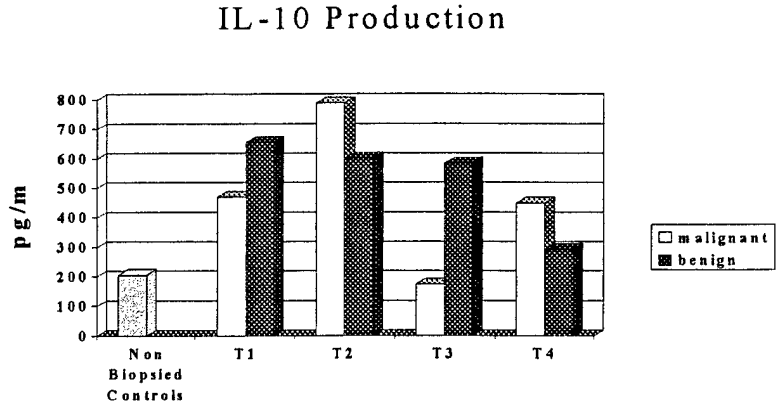

Figure 13 - Mononuclear cell production of IL-10 is depicted for non-biopsied control women and women pre (T1 and T2) and post (T3 and T4) breast biopsy. Peripheral blood mononuclear cells were activated by PMA/PHA and culture supernatants were collected at 48 hrs. IL-10 was determined by enzyme-linked immunoabsorbent assay. Bars represent the mean values. Standard error of the mean ranged from 103-190. N=8 for controls and N = 33, 36, 33, 34 for the benign group and N = 8, 12, 9, 10 for the malignant group.

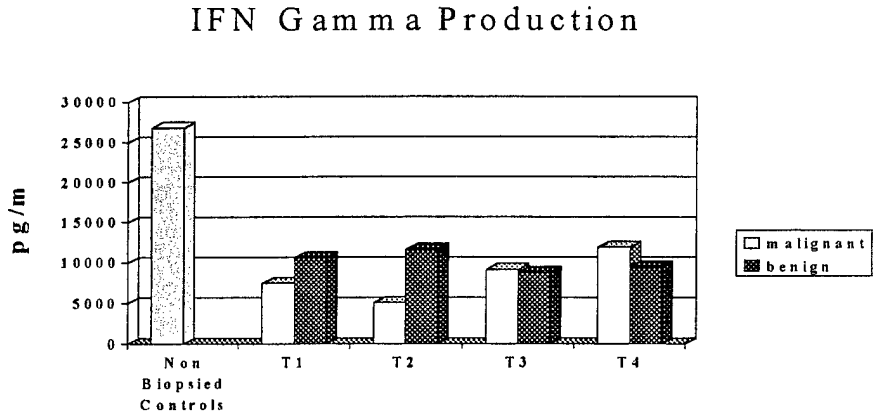

Figure 14 - Mononuclear cell production of IFN_γ is depicted for non-biopsied control women and women pre (T1 and T2) and post (T3 and T4) breast biopsy. Peripheral blood mononuclear cells were activated by PMA/PHA and culture supernatants were collected at 48 hrs. IFN_γ was determined by enzyme-linked immunoabsorbent assay. Bars represent the mean values. Standard error of the mean ranged from 1100-3300. N= 8 for controls and N = 34, 40, 32, 30 for the benign group and N = 11, 14, 9, 9 for the malignant group.

Serum samples were analyzed for cortisol and DHEA-sulfate. These results showed no significant differences between groups. Data not shown.

Discussion

Response to the experience of breast biopsy for all subjects. In order to determine whether psychological stress accompanies breast biopsy, scores on various stress measures pre and post biopsy were compared. The results clearly showed that women undergoing breast biopsy scored higher pre compared to post biopsy for essentially all of the psychological measures evaluated. There was no difference pre to post biopsy for the trait anxiety measure. This was anticipated in that this tool evaluates stable traits that would not be expected to change based upon a life event stress such as breast biopsy. Pre biopsy subjects experienced more total mood disturbance than did post biopsy patients. This was reflected in the POMS subscales with tension, anger, depression, and confusion decreasing from pre to post breast biopsy. Fatigue and vigor showed little change over time. The most dramatic changes were observed pre to post biopsy with the VAS BX tool. This tool evaluates acute stress and anxiety at the moment and the reduction pre to post for this measure indicates that the experience does represent an acute stressor.

A marked difference was observed in NKCA between control (non-biopsied subjects) and all women undergoing biopsy. Women entered into the study are all aware that breast biopsy is impending and as described above, experience psychological stress at T1. The impact upon this component of the immune system appears as early as T1 and continues throughout T4. As a whole, the psychological stress of biopsy is reduced at T3 and T4 for the women with benign biopsy results. However, the impact upon NKCA continues through time periods, T3 and T4. The reduction in NKCA was not accompanied by a change in the number of circulating NK cells. No difference in the number of circulating NK cells was observed between the normal controls or the biopsy patients, T1-T4. These data clearly suggest that the observed reduction in NKCA is a consequence of reduced NK cell activity and not a reduction in NK cell number.

Th1, Th2, and proinflammatory cytokine production falls into two categories for the biopsy patients. Either, production is increased for biopsy patients during all time periods (T1-T4) relative to control subjects or it is decreased. IL-6 is a pro-inflammatory cytokine that also falls into the former category. Its' production by all biopsy patients is greater than control subjects with increased levels of production, T1-T4. IL-4, and IL10 are also increased throughout the biopsy experience. In the latter category are IFNγ and IL-2. IFNγ is a Th1 cytokine and its' production by all biopsy patients was less than control subjects, with the least production of the cytokine observed pre biopsy. IL-2 also falls into the latter category with decreased production observed T1-T4, albeit a slight reduction compared to controls.

IL-2 is known to increase NKCA and NK cell proliferation (21) and IFNγ is known to enhance NK cell cytotoxicity (22). It is clear from the data that NKCA is decreased in this patient population, while IL-2 production is unchanged or possibly decreased. It should be noted that no circulating levels of cytokines were found in the serum of patients. Data not shown. However, IL-2 also stimulates lymphocytes to secrete cytokines, including IFNγ (14). Increased levels of this cytokine are known to increases NKCA, resulting in more efficient lysis of target cells and enhanced recruitment of pre-NK (16). The production of this cytokine is clearly decreased in these patients and may relate to the observed reductions in NKCA. Stress can down-regulate

NKCA and modulate IFNγ and IL-2 synthesis (23). Heightened levels of stress are related to decreased synthesis of IFNγ by lymphocytes from healthy subjects (24). A poorer NK response to IFNγ and IL-2 was observed in stressed individuals compared to non-stressed (25). Stress reduction interventions modulate NK activity and cytokine synthesis (26) suggesting an interactive relationship between stress and NK function that may be mediated in part by cytokines (27). Persons experiencing chronic stress have a more activated hypothalamic-pituitary-adrenal (HPA) axis compared to controls, demonstrable as chronically elevated levels of cortisol and catecholamines (28). This chronic activation may lead to greater concentrations of HPA products in lymphoid sites where NK cells reside and functional NKCA may be reduced. Alternatively, chronically activated NK may be less capable of stimulation to an effective anti-tumor state. Hence, stress activation of the HPA may down regulate NKCA by altering cytokine balance (e.g., decreased IFN-γ) and or responsiveness of NK cells to exogenous cytokines. It is noteworthy, that the stressed-induced increase in cortisol and decrease in DHEA shift the T-helper balance to a Th2 response, which does not support NKCA (13, 18). During stress, adrenal steroid hormone metabolism shifts such that DHEA/DHEAS production decreases and cortisol increases (18, 29). This shift in adrenal steroid hormone profile is recognized as having important immunomodulatory effects by altering Th1/Th2 cytokine balance. Cortisol decreases the production of IFNγ a Th1 cytokine. This has led to the concept that cortisol down-regulates Th1 cytokine production and promotes Th2 cytokine production (18). Conversely, DHEA appears to "buffer" or antagonize these effects of cortisol on cytokine production (29). Hence, it has been hypothesized that stress-induced changes in adrenal hormone secretion "drives the switch" from a Th1 to a Th2 immune response. Such a change in cytokine balance, characterized by low levels of IFNγ and IL-2, can depress NK cell function (15).

Response to the experience of breast biopsy for patients with benign compared to malignant breast biopsy findings. Benign patients were sorted from malignant patients and within group (pre to post biopsy) changes evaluated for all of the psychological tools. Group means for the pre biopsy sampling time periods were relatively consistent with no obvious differences between patients with benign compared to malignant findings. Prior to biopsy, patients who ultimately had benign findings tended to have numerically lower scores for the POMS TMD, all of the POMS subscales except fatigue and for the VAS BX when compared to patients who ultimately had malignant findings. The differences may not be significant and may be due to the fewer number of patients with malignant versus benign breast biopsy findings. No differences prior to biopsy were observed between these two groups for STAI, PSS, TRAIT, or the fatigue or vigor subscales of the POMS.

Marked decreases were observed in essentially all of the psychological tools from pre to post biopsy with the exception of TRAI. The greatest declines for the psychological assessments were observed in the benign group of patients, in particular for VAS BX and for the POMS TMD. For each of these two measures dramatic reductions were observed post biopsy compared to pre biopsy for women in the benign group. The results for the malignant patients also showed declines in overall score, but these declines were less dramatic than those for the women with benign findings. No attempt was made to further distinguish the malignant women (e.g. poor from good prognoses) and such distinctions may have influenced the data. Less dramatic differences were observed post biopsy for women with malignant compared to benign findings for STAI, PSS, and for the fatigue or vigor subscales of the POMS. In all cases the benign group tended to have numerical scores less than those of the malignant group of women. These data support the concept that breast biopsy is a stressful experience psychologically and that the psychological stress is greatest overall for the women with malignant findings. Yet, it is unclear why the total mood disturbance at T4 is greater for the benign group compared to the malignant. More importantly, our data show clearly that stress associated with the experience of biopsy can be quantified and that this quantification reveals differences both pre and post biopsy for malignant compared to benign patients.

Immunologically, interesting differences in NKCA between the benign and the malignant groupings were observed pre and post biopsy. At T1, T2, T3, and T4 a numerical increase in NK activity was observed for women who ultimately had benign findings compared to women who ultimately had malignant findings. In the malignant group, maximal NKCA was observed at T1. This activity was the maximal observed for any time period regardless of whether the patients were diagnosed with a malignancy or not. Mean NKCA for the malignant group reached the lowest observed mean activity at T4. In contrast, the group of women ultimately diagnosed as benign, showed NKCA 20 lytic units higher than the malignant group at T4. This activity suggests that the benign group was recovering NKCA at T4 while the malignant group's NKCA was diminishing. That is, the benign group appears to be returning to normal levels of NKCA while the malignant group's NKCA is diminishing. For both groups (either benign or malignant) maximal NKCA was at T1, suggesting that the experience of breast biopsy may in fact decrease overall NKCA regardless of the nature of the group. No differences were observed in the numbers of circulating NK cells for any of these time periods. Therefore it appears likely that changes in NKCA are a consequence of cellular activity only. It is tempting to speculate that the experience of breast biopsy reduces the overall capacity of patients to mediate tumor cell destruction (as judged by NKCA). When the immediate stressor is relieved, overall NKCA decreases. For the benign group of women, NKCA begins to slowly return to normal. For the malignant women, stress continues and NKCA continues to spiral down. Such a scenario would impact negatively this group of women and is fully consistent with this interim data analysis. The data of Andersen et al. substantiates stress-associated immune alterations in women with breast cancer (12). Those investigators found that stress was significantly related to decreased immune responsiveness and the data described herein is consistent with those results.

(6) KEY RESEARCH ACCOMPLISHMENTS:

- A psycho-immune assessment of women at two time periods pre and two time periods post breast biopsy has been accomplished.
- Stress, anxiety, and mood disturbance are heightened in women pre biopsy.
- Stress, anxiety and mood disturbance normalize in women post-biopsy who have benign results; however, women diagnosed with breast cancer have sustained elevations in stress, anxiety and mood disturbance post biopsy.
- Women diagnosed with breast cancer report higher levels of tension, depression, and confusion post-biopsy compared to women with benign breast biopsy findings.
- NK cell activity is depressed in women both pre and post breast biopsy compared to non-biopsied control women.
- Trends in cytokine alterations are emerging in women undergoing the experience of breast biopsy.

(7) REPORTABLE OUTCOMES:

One book chapter has been published and one manuscript have been accepted for publication. Both are included in the Appendix.

Witek-Janusek, L. and **H. L. Mathews**. Stress, Immunity, and Health. 2000. In: Handbook of Stress and Coping, V. Rice ed., Sage Publishing, pp. 47-68.

M. Nagabhushan, **H. L. Mathews**, and L. Witek-Janusek. Aberrant nuclear expression of AP-1 and NFkB in lymphocytes of women stressed by the experience of breast biopsy. Brain, Behav. Immun., In press.

Three abstracts have been published. The abstracts are included in the Appendix.

(8) CONCLUSIONS:

The evidence presented herein suggests that women experience increases in stress, anxiety, and mood disturbance when undergoing breast biopsy. This psychological disturbance subsides post-biopsy in those women who have benign breast biopsy results, but continues in women who have a diagnosis of breast cancer. Hence, the "stress" associated with breast cancer begins early, during the diagnosis phase of this cancer. Women who are psychologically stressed by the experience of breast biopsy have a decrease in NK cell tumoricidal activity compared to women who are not undergoing the experience of breast biopsy. Since NK cells mediate natural resistance against tumor cells and are particularly important in the control of tumor metastasis, the observed reductions in NK cell activity have implications for the control of cancer progression and hence the quality of life of women with breast cancer. Important regulators of NKCA are cytokines that are produced by subsets of lymphocytes and the relative balance of these cytokines can impact NK cell activity. Th1 lymphocytes produce IL-2 and IFN-γ. NKCA is promoted by these cytokines released by Th1 lymphocytes. Th2 lymphocytes produce IL-4, and IL-10. Th2 cells strongly support B lymphocyte activation and immunoglobulin production but contribute little to NK cell activity. Initial results indicate slight diminution in the overall production of these cytokines, but since they are produced at remarkably low levels it is less likely that they contribute to the balance between Th1 and Th2 effects on NK cell activity. IL-6 is produced by a variety of cell populations and is associated with inflammatory events. Out data shows clearly that IL-6 is markedly increased in its production during the assessed time periods. The significance of this observation remains unknown. However, as more patients are enrolled in the study it will be possible to develop a clearer understanding of the dynamic relationships between cytokines and NK cell activity in both malignant and benign populations of patients undergoing diagnosis for breast cancer.

Evaluation of Knowledge as a Scientific/Medical Product – These results provide evidence that the stress of cancer diagnosis, in this case breast biopsy, leads to prolonged periods of stress, anxiety, and mood disturbance which appear to be associated with depressed NK cell activity and an altered pattern of cytokine production. It appears as if stress-induced alterations in immunity are not transient but persist beyond the acute experience of the biopsy. This may be of particular relevance to women diagnosed with malignancy since they will be facing additional stressors related to cancer treatment and adaptation to illness. This emerging data set supports the need to incorporate stress-reduction strategies and to provide emotional support to women during the earliest stages of the cancer trajectory. Multi-disciplinary approaches to cancer diagnosis and treatment should seriously consider the incorporation of such approaches in the holistic care of women facing a cancer diagnosis. As this data supports, such approaches might prove to be beneficial to both the psychological and immunological status of women with cancer.

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(10) Appendices:

Abstract for the Seventh Annual International Cytokine Society Meeting

TH1/TH2 CYTOKINE PRODUCTION DURING THE STRESS OF BREAST CANCER DIAGNOSIS.
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Psychological stress can lead to changes in immune function. In this study, the experience of diagnostic breast biopsy was evaluated for its' effect, as a naturalistic stressor, on NK cell activity (NKCA) and cytokine production. A within group design in which women experiencing heightened psychological stress (pre biopsy) followed by alleviation of stress (post-biopsy) was employed. Psychological stress was measured by assessing mood disturbance, perceived stress, and anxiety. Most women experienced heightened perceived stress, anxiety, anger, depression, and tension prior to biopsy with alleviation post biopsy. Additionally, most women experienced decreased vigor and increased fatigue pre biopsy with alleviation post biopsy. Overall, these psychological effects corresponded with a reduction in NKCA pre-biopsy and an increase in NKCA post biopsy. Women with continued high mood disturbance and high stress post biopsy had even further reductions in NKCA. Women with high mood disturbance and high fatigue also had decreased capacities to produce interferon γ. Further, Th1/Th2 ratios of IL-2 or interferon γ : IL-10 showed that women with heightened perceived stress and fatigue had reduced ratios of Th1 to Th2 production of cytokines. These observations demonstrate this paradigm of human stress to result in not only an acute reduction in immune function at the time of breast biopsy but also a sustained reduction in NKCA and Th1 cytokine production with continued stress.

PSYCHO-ENDOCRINE-IMMUNE PROFILE: IMPLICATIONS FOR QUALITY OF LIFE IN BREAST CANCER PATIENTS

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Breast biopsy is an emotional experience and may impair anti-tumor immune responses. This study determined a woman's psycho-immune response pre (T1 and T2) and post (T3 and T4) breast biopsy. Stress, anxiety, and mood disturbance were heightened pre-biopsy in both benign and malignant patient groups. Post-biopsy, stress, anxiety, and mood disturbance normalized in the benign group but remained elevated in the malignant group. Natural killer cell activity (NKCA), a form of anti-tumor immune response, was depressed (T1-T4) in benign and malignant groups compared to non-biopsied control women. At T4, NKCA was lower in the malignant group compared to the benign group. IL-2, a Th1 cytokine, and IL-6, a proinflammatory cytokine, were increased from T1-T4 compared to control women. Conversely, IFNγ, a Th1 cytokine, and IL-4 and IL-10, Th2 cytokines, were depressed (T1-T4) relative to control women. Thus, breast biopsy produced stress, anxiety, and mood disturbance, which was relieved post-biopsy in women with benign results but sustained in women with malignancy. Associated with the stress of biopsy was depressed NKCA and altered cytokine production. In conclusion, psycho-immune dysregulation begins early in a woman's encounter with breast cancer and may influence cancer control. Thus, women may benefit psychologically and immunologically by stress-reducing interventions provided during the initial period of cancer diagnosis.

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PSYCHO-ENDOCRINE-IMMUNE PROFILE: IMPLICATIONS FOR QUALITY OF LIFE IN BREAST CANCER PATIENTS

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Breast biopsy is an emotional experience that may impair immune responses. This study determined a woman's psychological and immune response pre- (T1 and T2) and post- breast biopsy (T3 and T4). Stress, anxiety, and mood disturbance were heightened pre-biopsy in women experiencing breast biopsy. Post-biopsy, stress, anxiety, and mood disturbance normalized. Natural killer cell activity (NKCA) was depressed (T1-T4) in benign and malignant groups compared to non-biopsied control women. IL-2 and IL-6 production by peripheral blood mononuclear cells of biopsy patients was increased from T1-T4 compared to control women. Conversely, IFNγ, IL-4, and IL-10 were depressed (T1-T4) relative to control women. Thus, breast biopsy produced stress, anxiety, and mood disturbance, which was relieved post-biopsy in these women. Associated with the experience of biopsy was depressed NKCA and altered cytokine production. These changes in immune function continued well after stress, anxiety, and mood disturbance normalized in these women. In conclusion, psychological and immune dysregulation begin early in a woman's encounter with breast cancer and may influence cancer control. Thus, women may benefit psychologically and immunologically by stress-reducing interventions provided during the initial period of cancer diagnosis.

CHAPTER 3

Stress, Immunity, and Health Outcomes

Linda Witek-Janusek and Herbert L. Mathews

The assumption that psychological stress, physical stress, mood, and behavior modulate the immune system, and predispose an individual to illness, is centuries old. In the sixteenth century, the Greek physician Galen observed that melancholy women were more predisposed to the development of tumors. Today, the assumption is widely held that stress, emotions, and behavior affect health, well-being, and predisposition to disease. For example, a character proclaims in Woody Allen's film *Manhattan,* "I can't express anger, I grow a tumor instead." Only recently, however, has this mind-immune relationship been subjected to rigorous scientific inquiry.

The organized establishment of the science of psychoneuroimmunology is often credited to Robert Ader, who first introduced this term in his presidential address to the American Psychosomatic Society (Ader, 1980). Ader defined *psychoneuroimmunology* as the study of the interactions among behavioral, neural, endocrine (neuroendocrine), and immunological processes of adaptation. The central premise is that an individual's response and adaptation to the environment is an integrated process involving interactions among the nervous, endocrine, and immune systems. This is in contrast to the traditional view of the immune system in which it is autonomous and functions independently of the other organ systems of the body. Today, psychoneuroimmunology is a multidisciplinary science that includes nurses, psychologists, immunologists, microbiologists, neuro-

AUTHORS' NOTE: This chapter is dedicated to the memory of my (LWJ) mentor and my friend, Dr. Sabath F. Marotta (1929-1996), who introduced me and numerous other nurses to scientific inquiry and stress physiology. May his memory live on in our collective contributions to the field of stress. This work was supported in part by the Department of the Army (DAMD-98-8120), the National Cancer Institute (CA-77120-01), the National Institute of Nursing Research (NR-00085), the National Institute of Allergy and Infectious Disease (AI-31127), Catholic Health Partners, and the Cancer Federation. The expertise of Josh Takagishi and Maribel Barrigan is gratefully acknowledged. The content of this chapter does not reflect the position or the policy of the Department of the Army or the U.S. government.

scientists, endocrinologists, and others. The collective aims of these scientists are to explore and explain the common belief that one's behavior and emotions can influence stress, immunity, and health outcome.

Despite the recent development of psychoneuroimmunology as a discipline, initial evidence that linked stress to the immune system was reported by Hans Selye in the 1930s. In his general adaptation syndrome, Selye described a triad of responses to acute physical stress that consisted of adrenal gland enlargement, gastric erosion, and thymic involution (Selye, 1936, 1976). Since then, scientific evidence confirming biological links among the nervous, endocrine, and immune systems has accumulated. These links include direct innervation of lymphatic tissue by the central nervous system and a shared communication network in which cells of the nervous, endocrine, and immune systems use common molecules and receptors to reciprocally modulate biologic activity. Thoughts, emotions, and behavior are known to activate anatomical and biochemical pathways, and these pathways in turn modulate immune function (La Via & Workman, 1998). Such observations and demonstrations have permitted advocates of psychoneuroimmunology to suggest that biobehavioral interventions aimed at strengthening immunocompetence may be an important component of holistic health care (Kiecolt-Glaser & Glaser, 1992).

➢ NEURAL-IMMUNE INTERACTIONS

The connection between the brain and the immune system is through direct innervation of lymphoid tissue and through the release of products from the brain that bind to membrane receptors on immunologically competent cells. It is clear that primary and secondary lymphoid tissues are innervated with noradrenergic and peptidergic nerve fibers (Felten et al., 1987). The Feltens's immunohistochemical studies provide direct evidence of the close association between presynaptic sympathetic nerve endings and lymphocytes and macrophages (Felten, Felten, Carlson, Olschowka, & Livnat, 1985). Experimentally produced brain lesions of the hypothalamus, hippocampus, and cerebral cortex alter immune function, suggesting a neural-immune interactive network of connections. Those areas of the brain that exert immunomodulatory effects are areas concerned with emotions and with visceral, autonomic, and neuroendocrine regulation, thus establishing the "hardwiring" between neural centers that process emotions and immune cells. Further verification of a neural-immune network or axis was provided when lymphocytes and macrophages were shown to bear receptors for adrenergic substances (both α- and β-adrenergic receptors) and various neuropeptide hormones, including vasoactive intestinal polypeptide, somatostatin, calcitonin gene-related peptide, substance P, and opioids (Stevens-Felten & Bellinger, 1997). The presence of such receptors on immune cells provides a mechanism whereby the immune system can respond to biochemical signals from the brain. Activation of these receptors leads to functional changes in immune response (i.e., lymphocyte proliferation, cytotoxicity, antibody production, and cytokine secretion).

A pivotal step in firmly establishing that the brain and immune system interact was accomplished by psychologists who, using animal models, demonstrated that classical psychological (Pavlovian) conditioning could produce immunologic changes (Ader & Cohen, 1993). Such conditioning and its effect on the immune system have been demonstrated clinically. For example, research has documented the occurrence of anticipatory immunosuppression prior to the administration of chemotherapy (Bovbjerg et al., 1990; Fredrikson, Furst, Lekander, Rothstein, & Blomgren, 1993).

Investigators continue to unravel the intricate interplay among the nervous, endocrine, and immune systems. The associated immunologic changes that occur in response to neuroendocrine mediators, however, are

highly complex, and often the characterization of putative interactions has been measured only *in vitro,* in which one variable is manipulated. *In vivo,* however, immunologically competent cells respond to multiple stimuli, including numerous so-called molecules of emotion, within a microenvironment. Ultimately, the net immune response is an integration of these stimuli. The multiple levels and complexity of such immune modulation are remarkable, considering that numerous peptide and hormonal mediators can augment and diminish immune function (Wang, Fiscus, Yang, & Mathews, 1995; Witek-Janusek & Mathews, 1999a). It remains to be determined how these peptides and mediators fit within a homeostatic framework or are altered by environmental perturbation.

Adding additional complexity, it is well established that not only do nerves and secretory products from the brain influence immune function but also the converse is true. Immune activation can modulate central nervous system activity. Hugo Besedovsky and collaborators conducted seminal studies, which demonstrated that antigenic challenge of the immune system can produce an increase in neural firing within the medial hypothalamus (Besedovsky, Felix, & Haas, 1977). A peak immune response was associated with a decrease in norepinephrine turnover. Cytokines produced by antigen-activated lymphoid cells altered the turnover of norepinephrine (Besedovsky, del Rey, Prada, Burri, & Honegger, 1983).

It is now understood that alterations in cytokine secretion subsequent to immune activation mediate behavioral effects often associated with illness. For example, interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) mediate sickness behavior (the fatigue, lethargy, and decreased appetite associated with infectious illness) (Dantzer et al., 1998). Because they are large protein molecules, cytokines do not readily cross the blood-brain barrier. They are believed to signal the brain by entering neural structures that do not possess tight capillary endothelial barriers, such as the organum vasculosum laminae terminalis and area postrema. In addition, recent evidence indicates that cytokines released in the periphery can activate sensory afferents, such as vagal afferents, and signal central nervous system (CNS) areas involved in immune-related behavioral responses (Watkins, Meier, & Goehler, 1995).

Collectively, this evidence supports the concept of a dynamic neuroendocrine-immune network whereby soluble products of immunologically competent cells affect the CNS following antigenic challenge. It is this conceptualization that led Blalock to liken the immune system to a sensory organ capable of informing the CNS of an antigenic challenge (Blalock & Smith, 1985).

Both psychological and physical stressors are known to activate neuroendocrine pathways that interact with the immune system (Chrousos, 1998). Stressor activation leads to increased secretion of neurosecretory hormones from the hypothalamus, such as corticotropin-releasing hormone (CRH). In turn, these hypothalamic hormones regulate secretion of pituitary hormones, such as adrenocorticotropin hormone (ACTH) and endorphins. Because there are shared hormonal receptors on cells of the immune and neuroendocrine systems, reciprocal interactions between these systems are possible (Reichlin, 1993; Weigent & Blalock, 1999). Neuroendocrine secretory products have immunomodulatory effects and alter leukocyte function (e.g., the immunologic effects of glucocorticoids, endorphins, ACTH, growth hormone, and prolactin). These effects include the regulation of cytokine secretion, antibody synthesis, natural killer cell (NK) activity, and lymphocyte proliferation (Weigent & Blalock, 1999). The complex interactions between the neuroendocrine and immune systems are believed to, in part, downregulate inflammatory responses and limit continuous proliferation of lymphoid cells or excessive production of immune cell products or both (Munck & Guyre, 1986).

Interestingly, neuroendocrine hormones can be produced by leukocytes, the most well studied of which is proopiomelanocortico-

tropin (POMC). POMC is a precursor molecule for the hormones ACTH and endorphin. Although the role of hormones produced by immune cells is under investigation, it is likely that they function by autocrine/paracrine mechanisms within the local lymphoid microenvironment (Weigent & Blalock, 1999). The finding that immune cells can produce hormones normally secreted from the anterior pituitary emphasizes the close relationship of the immune and endocrine systems. Finally, immune cell secretory products (e.g., cytokines) alter neuroendocrine cell secretion. For instance, cytokines have actions at both the hypothalamic and pituitary levels. Cytokines, such as IL-1, IL-2, IL-6, and TNF, activate the adrenal axis, whereas IL-1 and TNF inhibit the gonadal axis and TNF and interferon-gamma (IFN-γ) suppress the thyroid axis (Weigent & Blalock, 1999).

The bidirectional nature of the neuroendocrine and immune systems likely accounts for the effect of stress on the immune system (Figure 3.1). Regulatory hormones and neuropeptides once believed to be confined to the brain or endocrine system or both are now known to be mutually expressed by all three systems (nervous, endocrine, and immune), and as a result each system may be capable of modulating the function of the other.

In summary, during the past 15 years empirical evidence has emerged that supports the existence of a communication network linking the nervous, endocrine, and immune systems. Psychological stimuli modulate the immune response either through direct activation of neural pathways that terminate in lymphoid tissue or by activation of neuroendocrine circuits leading to the release of molecules that bind to immunologically competent cells. Conversely, the immune system recognizes noncognitive stimuli, such as bacteria, fungi, and viruses, resulting in the secretion of an array of cytokines that act on receptors of the neuroendocrine system. Collectively, cognitive and noncognitive stimuli form a network, which is the basis for behaviorally induced alterations in immune function (Weigent & Blalock, 1999). It is likely that this neuroendocrine-immune network mediates the effect of stress on the development or progression or both of immune-based disease.

➢ STRESS AND IMMUNITY

Stressful life events, and the subsequent emotional and behavioral responses to these events, are commonly believed to alter immunity. When external demands (i.e., stressors) exceed an individual's adaptive capabilities, a stress response ensues (Lazarus & Folkman, 1984). It is the subsequent neurological and endocrinological changes that are believed to produce stress-elicited immune alterations. Studies during the past decade provide convincing evidence that psychological stress can affect the immune system (e.g., lymphocyte proliferation, NK activity, antibody synthesis, and cytokine production). These studies have been accomplished with animal models and in human stress situations, including both experimentally produced stress and naturalistic paradigms for stress evaluation. This chapter focuses on the major human stress paradigms.

Early studies supporting the effects of stress on immunity were conducted by the research team of Glaser and Kiecolt-Glaser. These investigators conducted a series of stress studies in medical students that demonstrated the immunosuppressive effects of in-class examinations (Kiecolt-Glaser, Garner, Speicher, Penn, & Glaser, 1984). The results of these studies indicate that the stress that accompanies examinations leads to a wide range of immunosuppressive effects, including decreased NK cell activity (Glaser, Rice, & Speicher, 1986), lymphocyte proliferation (Glaser et al., 1987), IFN-γ production (Glaser et al., 1986), IL-2 production (Glaser et al., 1990), and latent viral activation as evidenced by increased antibody titer to the virus (Glaser et al., 1992).

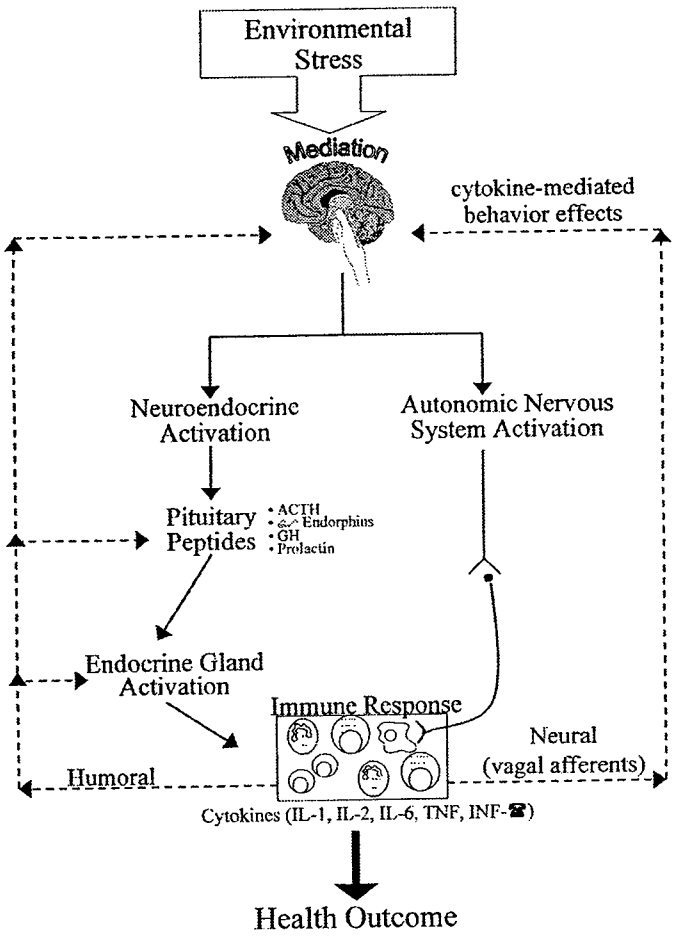

Figure 3.1. Summary and possible interconnections by which environmental stimuli, or stress, can affect the immune response and health outcomes. Perceived environmental stress is mediated by the central nervous system and can lead to neuroendocrine and autonomic nervous system activation. As a result, the immune response can be altered by autonomic nerve fibers that directly synapse with immune cells and by circulating catecholamines released from the adrenal medulla. In addition, further alteration can be produced by secretory products (hormones and neuropeptides) released from the pituitary and endocrine target glands (adrenal cortex, thyroid, ovaries, and testes). In turn, feedback (dashed lines) from immune cell products (cytokines) can modulate endocrine and central nervous system activity by either humoral or neural communication networks.

Furthermore, medical students with lower anxiety levels had faster and stronger immune responses to hepatitis B vaccination than did students with higher levels of anxiety (Glaser et al., 1992; Glaser, Kiecolt-Glaser, Malarkey, & Sheridan, 1998). Recently, examination stress was shown to alter cytokine production that shifts the cytokine pattern away from a Th1 to a Th2 type of response (Maes et al., 1998). This shift is characterized by a decrease in secretion of IFN and an increased secretion of IL-10. The authors suggest that the shift in cytokine production may partially explain the increased incidence of

viral infection, latent viral expression, allergic and asthmatic reactions, and autoimmunity reported during times of high stress (Marshall et al., 1998).

The type of immune response seen as a result of stress is dependent on the acute versus chronic or repeated nature of the stressful event (McEwen, 1998). For example, acute stressors, such as parachute jumping, are correlated with a mobilization in the numbers of NK cells; this is likely attributable to a change in cell trafficking related to adrenergic arousal (Schedlowski et al., 1993) or glucocorticoid secretion (McEwen, 1998) or both. Studies such as these suggest that acute stress produces a redistribution of lymphocytes and macrophages in the body. These cells marginate on blood vessel walls and compartmentalize in the skin, lymph nodes, and bone marrow. It is theorized that acute stress activates the immune response and prepares the organism for potential encounters with an immunologic challenge. This activation may exacerbate autoimmune or allergic responses (Dhabhar, Miller, McEwen, & Spencer, 1996). Repeated or chronic stress, however, suppresses immune responsiveness, particularly cell-mediated immunity, and increases susceptibility to infectious challenge and tumor cells (McEwen, 1998). Chronic stressors, such as bereavement, caregiving, marital conflict, and divorce, impair the ability of NK cells to be lytic and to respond to cytokines (IFN-γ or IL-2) *in vitro* (Esterling, Kiecolt-Glaser, & Glaser, 1996; Herbert & Cohen, 1993; Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991; Kiecolt-Glaser, Glaser, Cacioppo, & Malarkey, 1998). Other aspects of cellular immunity are also affected by chronic stress (Herbert & Cohen, 1993), including decreased lymphoproliferation, NK cell activity, numbers of circulating lymphocytes, as well as salivary and serum immunoglobulin levels.

The impact of chronic stress has been poignantly illustrated by assessing the immune response of individuals caring for relatives with Alzheimer's disease. Kiecolt-Glaser et al. (1987) found that such caregiving was accompanied by greater distress and heightened levels of herpes virus-specific antibody (suggesting viral reactivation). Furthermore, elderly individuals experiencing the chronic stress of caring for a spouse with Alzheimer's disease had attenuated responses to the influenza vaccine and more physician-confirmed respiratory infections than control subjects. Health behaviors did not differ between the two groups.

Conversely, Irwin et al. (1991) reported no differences in NK cell activity between caregivers and control subjects. Esterling et al. (1996), however, found that both caregivers and former caregivers (those whose relative had died at least 2 years previously) had blunted NK cell activity compared to nonstressed control subjects. Interestingly, the results of this study suggested that the psychological and immunological aftermath of caregiving persists beyond the actual stressful experience. In an attempt to reverse the immunosuppressive effects of stress in the elderly, these investigators enrolled subjects in a 1-month stress-reduction program that used progressive muscle relaxation. This form of stress reduction produced a 30% increase in NK cell activity (Kiecolt-Glaser et al., 1985).

The type and magnitude of stress-elicited effects on the immune system are influenced by many factors. Such factors may relate to the stressor, such as the type, intensity, and duration of the stressful stimulus. The sampling time frame between the stressor and the immune response can also influence whether an effect can be measured. Furthermore, not all components of the immune system may respond to a stressor. Therefore, it is important that the immune parameter to be measured be carefully chosen within the context of the population or illness studied or both. A variety of host or subject factors will also influence the immune response to stress, such as age, preexisting illness, nutritional status, substance abuse, exercise habits, adequacy of sleep, coping, and social support (Kiecolt-Glaser & Glaser, 1988; Zeller, McCain, McCann, Swanson, & Colletti, 1996).

The primary criticism of many stress-immune studies is that although the immune change observed is often statistically signifi-

cant, the magnitude of the change is small and often within normal limits. Whether or not such a change in immune function is significant to health outcomes remains to be determined. There are studies, however, that have found that stress-induced immune changes can increase susceptibility to infectious disease and may also influence the course of disease (Cohen, Tyrel, & Smith, 1991; Spiegel, Bloom, Kraemer, & Gottheil, 1989). Such studies support the contention that even small changes in immune function may have health-related significance.

➢ STRESS-IMMUNITY AND HEALTH OUTCOMES

One fundamental question that remains unanswered in the field of psychoneuroimmunology is whether or not stress-induced alteration in immune function plays a role in disease development or disease progression or both. Numerous studies, although inconclusive, have shown stress to influence the course or progression of illness or disease (e.g., cancer, infectious disease, and HIV). Few studies, however, provide definitive evidence that links stress, immunity, and health outcomes. This area remains a challenge for researchers in psychoneuroimmunology.

One of a handful of well-controlled studies that examined the effect of psychological stress on susceptibility to illness was conducted by Cohen et al. (1991). They investigated the relationship between stress and the common cold using a viral challenge paradigm. Following extensive health and psychological assessment (for the previous 12 months), 394 volunteers were randomized to receive either a low infectious dose of a respiratory virus or saline. For 2 days prior to viral challenge and 7 days postchallenge, volunteers were quarantined. Rhinovirus infection was based on the development of clinical symptoms of a cold, the development of virus-specific antibodies, and the culture and isolation of the inoculated virus. The results revealed that psychological stress predicted susceptibility to colds among the initially healthy people exposed to the respiratory virus. Psychological stress was operationalized as an index of the number of negative life events, the perceived impact of these negative life events, perceived stress, and negative affect. In a related study, Cohen et al. analyzed the relationship of an individual's social contacts to the development of the common cold. In 276 volunteers exposed to rhinovirus, a greater resistance to upper respiratory infection was exhibited in subjects who had the greatest diversity of social contacts (friends, family, and community). Interestingly, greater resistance to infection was related to increased numbers of social contacts and not to the absolute number of individuals involved in the social contacts (Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997). Recently, these investigators reported that acute stressful life events (less than 1 month in duration) were not associated with the onset of colds. Severe chronic stressors (1 month or longer in duration), however, were associated with the risk of cold development. The most prevalent chronic stressors for this study group were under- or unemployment or enduring interpersonal difficulties with family or friends (Cohen et al., 1998).

➢ STRESS AND WOUND HEALING

Studies of the effects of stress on wound healing and tissue repair have suggested that stress-induced neuroendocrine activation impairs healing and delays recovery. Both animal and human models of wound healing have been used to examine the effects of stress. In one study (Padgett, Marucha, & Sheridan, 1998), the effects of restraint stress on the healing of a sterile punch wound in rats were studied. Rats were subjected to restraint stress for 3 days prior to and for 5 days after wounding. Wound healing was measured using photography and image analysis. Compared to control rats, which were wounded but not restrained, healing was delayed an average of 3 days in the restraint stressed group. Treatment of the restraint stressed group with a glucocorticoid receptor

antagonist produced healing rates that were similar to those of control animals. These results demonstrate that restraint stress delayed wound healing. Because the glucocorticoid antagonist reversed this effect, the delay was likely due to a stress-induced increase in glucocorticoids. Padgett et al. (1998) hypothesized that the stress-induced elevation in glucocorticoids prevented the early part of wound healing in which macrophages move into the area to remove cellular debris and secrete growth factors, cytokines, and chemotactic factors needed for tissue repair. Glucocorticoids are well-known to suppress the inflammatory response, including the production of IL-1, IL-6, and TNF-α (Bendrups, Hilton, Meager, & Hamilton, 1993). The results of this study provide compelling evidence, albeit in an animal model, that disruption of neuroendocrine homeostasis by a stressor modulates wound healing.

The ability of stress to delay wound healing has also been shown in human stress paradigms. Kiecolt-Glaser, Marucha, Malarkey, Mercado, and Glaser (1995) studied the effects of chronic stress on caregivers (spouses) for patients suffering from Alzheimer's disease. Punch biopsy wounds were applied to caregivers and age-matched control subjects. The results indicated that wound healing was markedly delayed in the caregivers compared to control subjects. These differences were not related to other covariates, such as nutrition, sleep, or the presence of other illnesses. In another study, wound healing was delayed by the more acute and benign stress of academic examinations. Two punch biopsy wounds were placed on the hard palate of dental students during summer vacation and then on the contralateral side 3 days prior to the first major exam of the term. Mucosal wound healing took 3 days longer to complete during the exam period. The production of mRNA for IL-1β was also reduced during the stress of examination (Marucha, Kiecolt-Glaser, & Favagehi, 1998).

In summary, the previously discussed studies provide compelling evidence suggesting that stress can impair tissue repair and wound healing. This can have significant implications for recovery from injury and surgery, especially in vulnerable populations, such as individuals with diabetes, impaired tissue perfusion, and advanced age. Delayed healing of wounds increases the risk for wound complication by infectious pathogens, which can further prolong recovery and length of hospital stay. Nurses are in a pivotal position to recognize and reduce stress and to teach stress management skills. This has the potential to promote both healing and recovery and enhance health outcomes.

➢ STRESS-IMMUNITY AND CANCER

The immune system is believed to play a role in surveillance against malignantly transformed cells. It has been hypothesized that stress-induced suppression of immune cell activity (e.g., NK cell activity) may alter the clinical course of cancer. The relationship between NK cell activity and cancer is complex (Rosenberg & Lotze, 1986). NK cells, however, are also important in the control of viral infections (Trinchieri, 1989; Whiteside & Herberman, 1989). As such, NK cells may prevent the development of infectious complications in cancer patients who are often immunosuppressed.

There are a few highly intriguing studies that have examined the relationship between stress and immunity in cancer patients. Levy et al. (1990) found that estrogen receptor status predicted NK cell activity in 66 women with Stage I or II breast cancer 3 months after surgery with or without adjuvant therapy. These researchers also showed that social support contributed significantly to a regression model predicting higher NK cell activity. That is, the greater an individual's social support, the higher the individual's NK cell activity.

Andersen et al. (1998) studied stress-immune parameters in 116 women who were diagnosed with invasive breast cancer (Stages II and III). Women were enrolled within 4 months of their breast surgery but prior to

adjuvant therapy initiation. Stress was measured using the Impact of Event Scale, which is a self-report measure of intrusive and avoidance thoughts and behaviors (Horowitz, Wilner, & William, 1979). Using hierarchical multiple regression analysis, their results revealed that higher stress levels significantly predicted lower NK cell activity, diminished NK cell response to IFN-γ, and decreased lymphocyte proliferation (Andersen et al., 1998). It is noteworthy that this study controlled for extraneous variables that might also affect immunity, including age, stage of disease, nutritional status, and days since surgery. The results are intriguing and suggest that stress may play a pivotal role in women with cancer, possibly resulting in more susceptibility to cancer progression or infectious complications or both.

Researchers have begun to address the definitive question as to whether psychosocial interventions can produce health effects that slow cancer progression and promote survival (Fawzy, Fawzy, Arndt, & Pasnau, 1995; Greer & Brady, 1988; Speigel, 1996). Randomized prospective trials have shown protective effects of psychosocial interventions on cancer progression (Spiegel, Sephton, Terr, & Stites, 1998). Fawzy et al. (1993) studied the effects of a behavioral intervention in patients with malignant melanoma. Subjects were randomized to an intervention consisting of six 90-minute sessions including health education, stress management, coping skills, and group discussion. Six months later, the intervention group showed reduced psychological distress and enhanced immune function (increased IFN-α and augmented NK cell activity) compared to the nonintervention group. Although no association between survival and NK cell activity was found, individuals with higher baseline NK cell activity had a decreased incidence of disease recurrence.

In another study, the effects of a home visit and educational intervention program for lymphoma and leukemia patients were investigated (Richardson, Shelton, Krailo, & Levine, 1990). The results showed that patients in the intervention group were more compliant with their medical treatment. More important, when controlling for this difference, members of the intervention group lived significantly longer than members of the control group.

A landmark study that assessed the effect of behavioral intervention on cancer survival was conducted by David Spiegel and colleagues (1989). They reported compelling results suggesting that an intervention, characterized as supportive-expressive group therapy, increased the survival of women with advanced breast cancer. Fifty of 86 women with advanced breast cancer were randomly assigned to support groups. The groups were designed to build strong supportive bonds, encourage "emotional expressiveness" about cancer, confront fears of dying and death, reorder life's priorities, improve relationships with family and friends, enhance communication with and development of shared problem solving with physicians, and teach self-hypnosis for pain control (Spiegel et al., 1998). The women were followed for 10 years, and a significant 18-month increase in survival for women in the intervention group was observed. Further analysis of the results of this study, in which medical records were reviewed, showed no difference in therapeutic treatment that could account for the differences in survival. Rather, a correlation was found between group support and survival. Spiegel's research team is currently replicating this study with a larger group in which endocrine markers of stress and cellular immune response, including NK cell activity, are being measured in addition to survival. It is hypothesized that psychosocial support will buffer the immunological consequences of cancer-associated stress and thereby improve disease outcomes (Spiegel et al., 1998).

In addition to the ongoing study of Spiegel, Andersen and colleagues (1998) are conducting a prospective, randomized study evaluating the effectiveness of stress-reduction interventions on psychological, immunological, and survival outcomes in women with advanced breast cancer. The structured intervention includes several stress-reduction strat-

egies, such as progressive muscle relaxation, social and emotional interventions designed to increase the quality of life, and healthy living habits. The intervention is provided weekly for the first 4 months and monthly for an additional 8 months. Psychological and immunological variables are being measured, with survival being the ultimate end point of this ongoing longitudinal study (McNeil, 1998; Voelker, 1997).

The role of psychological stress in cancer progression or response to treatment or both remains controversial, as was expressed in an editorial by Cohen and Rabin (1998). They contend that it is not clear if the effects of behavioral interventions are due to an individual's greater adherence to a healthy lifestyle or to the behavioral intervention therapy or both. The results of behavioral-based intervention studies are highly provocative and difficult to ignore, however. Indeed, the results of the ongoing clinical trials will provide further data that will aid in the understanding of the importance of stress, its impact on the immune system, and cancer control.

➢ STRESS-IMMUNITY AND HIV

Individuals living with HIV face numerous stressors, such as family discord, change in occupation, economic hardship, social isolation, and bereavement (McCain & Zeller, 1996; Robinson, Mathews, & Witek-Janusek, 2000). Because the immune system plays a dominant role in the prevention of viral infections and in the suppression of latent viral infections, stress-induced changes in immune function may alter disease progression. Evidence suggests that stress-induced modulation of immunity may alter the course of HIV infection (McCain & Zeller, 1996; Robinson et al., 2000). Psychological variables are hypothesized to mediate host resistance to the HIV virus by modifying behavioral practices and by promoting an optimal neuroendocrine and immune milieu. Overall, most of these studies are fraught with methodological difficulties, such as small and nonhomogeneous samples, lack of control for treatment and disease stage variables, inability to document or measure the presence of psychosocial stress in the sample, and lack of sensitive and relevant indices of immune measures. Nevertheless, the results are intriguing.

Goodkin, Fuchs, Feaster, Leeka, and Rishel (1992) studied stress-immune correlates in asymptomatic HIV-positive males. Although the sample size was small, the results suggested that men with a lower ability to cope with stress had lower total lymphocyte counts, whereas men with higher coping abilities had greater numbers of CD4+ T lymphocytes. A series of stress-immune studies have originated from the University of Miami's Center for the Biopsychosocial Study of AIDS; some of these studies have evaluated the psychoimmune effects of the stress of HIV antibody testing (i.e., test notification stress). This research team reported a significant relationship between increased anxiety (State Trait Anxiety Index [STAI]) at the time of notification of test results and decreased NK cell activity. No association with lymphocyte proliferation was found (Ironson et al., 1990).

In a similar study, during a 5-week period before and after HIV testing, seropositive subjects reported higher anxiety (STAI), higher depression, increased intrusive thoughts, and lower lymphocyte proliferation rates than seronegative subjects. Although plasma cortisol levels declined significantly in the seropositive group during the study period, they were within normal limits (Antoni et al., 1991). McCain and Cella (1995) found a significant relationship between high stress and lower CD4+ cell numbers in their study of a heterogeneous group (heterosexuals, minorities, injecting drug users, and those with various stages of disease progression) of 53 men with HIV disease. These same investigators examined the effect of a stress management intervention in HIV-positive individuals. Although a reduction in stress was demonstrated, they failed to show any significant ac-

companying change in immune function (McCain, Zeller, Cella, Urbanski, & Novak, 1996). In another intervention study, however, Esterling and colleagues (1992) measured antibody titers to Epstein-Barr virus (EBV) as the immune end point. Both HIV-positive and HIV-negative men in the 10-week program had significant decreases in anti-EBV viral encapsulated antigen when compared to their matched controls (Esterling et al., 1992). Because of the intriguing nature of these intervention studies, similar lines of research will likely be pursued in the future.

Although there is no clear mechanism for how stress influences HIV disease progression, Clerici and colleagues (1994) proposed an "immunoendocrinological" hypothesis implicating the potential role of elevated cortisol in the progression of HIV disease through modulatory effects on viral replication, cytokine modulation, and increased induction of apoptosis. Supportive evidence for this theory has been provided by reports that cortisol enhances HIV viral infections when added to cell culture medium containing human lymphocytes (Markham, Salahuddin, Veren, Orndorff, & Gallo, 1986) and HIV viral replication when added to monocyte cultures (Swanson, Zeller, & Spear, 1998). Norepinephrine, a major catecholamine released during stress, also accelerates HIV replication (Cole, Korin, Fahe, & Zack, 1998).

It is likely that studies examining psychoneuroimmune parameters in HIV disease are limited by the immune outcome variables measured. It is possible that psychological effects may not have a measurable impact on indices of HIV disease development or progression or both. More important, stress may play an important role in the HIV-infected person's susceptibility to opportunistic infection. Consequently, there is a need to design and implement studies aimed at determining the role of psychological stress on immune system indices designed to measure defense mechanisms important in host defense against opportunistic infection (Robinson et al., 2000). The nature of the stress-immune relationship in HIV disease needs to be carefully evaluated within the context of currently used antiretroviral therapy and within the context of future therapeutic approaches. Such therapies may not only alter immune responsiveness in those with HIV but also influence the type of stress they encounter as they live with HIV.

➢ STRESS-IMMUNITY AND INFECTION

Vulnerable populations, such as cancer patients and persons with HIV, face a multitude of stressors. These stressors can influence the immune system and increase susceptibility to infectious diseases. Psychological stress seems to alter the susceptibility of individuals to infectious agents and influences the onset, course, and outcome of the pathology associated with infection (Biondi & Zannino, 1997). Moreover, infectious disease can be a stressor. The human body's response to infection and to immunological challenge resembles both physical and psychological stress (Dunn, Powell, Meitin, & Small, 1989).

Infection can activate the hypothalamic-pituitary-adrenal axis (HPA) axis and increase the synaptic release of norepinephrine and serotonin in the brain (Dunn, 1993). Thus, by physiological criteria, infection can be regarded as stressful. The activation of the HPA axis associated with immune responses has been interpreted as a signal to the brain indicating the presence of an infectious threat from the external environment, triggering a stress response (Blalock & Smith, 1985). Once an effective immune response has been initiated, the HPA axis is thought to negatively regulate the immune system by the release of glucocorticoids that limit the inflammatory response and prevent overreactivity and autoimmune phenomena (Besedovsky, del Rey, Sorkin, & Dinarello, 1986; Munck & Guyre, 1986). Thus, the effects of stress on the immune system and the effects of the immune system on the neurologic response to infection are a complex and interrelated series of

physiologic events with many reciprocal interactions. Many specific infectious states appear to have a clear association with stress.

Tuberculosis

Stress has long been associated with the pathogenesis of tuberculosis. With the recent resurgence of tuberculosis, understanding the potential role of stress in susceptibility to and progression of this infectious disease has become even more important. In previous studies, high rates of tuberculosis have been reported among socially isolated individuals and in schoolchildren and their teachers during periods of emotional stress, such as during war (Guyre, Girard, Morganelli, & Manganiello, 1988; Ishigami, 1919). These studies showed a reduced capacity of the infected individuals to phagocytize the infectious agent and suggested that stressful situations might serve as cofactors in the development of tuberculosis. Until recently, very little evidence existed to support this suggestion. Work using experimental animals has shown that HPA axis activation, induced by restraint stress, increased the growth of the tubercle bacillus (Zwilling et al., 1990). Adrenalectomy and treatment with the glucocorticoid receptor antagonist RU486 abrogated this effect. Furthermore, HPA axis activation suppressed phagocyte function and decreased the capacity of the animals to produce immune augmenting cytokines in response to the mycobacteria (Brown, Sheridan, Pearl, & Zwilling, 1993).

The effects of stress in experimental animals may have important implications in human disease. In an extensive study, tubercular patients were shown to have a dramatic increase in the number of stressful life events approximately 2 years prior to their hospitalization (Homes, Hawkins, Bowerman, Clarke, & Joffe, 1957). Likewise, mortality due to tuberculosis has been shown to be higher in subjects who have experienced divorce (Somers, 1979). It is possible that the reactivation of tuberculosis may be a consequence of susceptible populations being affected by stressors. Stress, mediated by neuroendocrine-immune interactions, may significantly contribute to this infectious disease, which continues to be a serious health hazard worldwide.

Viral Infections

Colds and influenza have been useful models to evaluate the role of psychoneuroimmunology in human disease. As discussed previously, Cohen et al. (1991) evaluated the significance of psychosocial factors on the common cold. Subjects were inoculated with respiratory viruses, and the risk of developing the infectious disease was directly associated with chronic stress. This study and many others showed similar effects of stress on the development of colds and influenza but no direct effect of stress on the immune system of the more susceptible individuals (Clover, Abell, Becker, Crawford, & Ramsey, 1989).

Many other studies have evaluated the effects of stress on latent viral infections caused by herpes simplex virus, EBV, and HIV. These viruses are typically latent in humans, and the hypothesis that stress favors viral reactivation has been evaluated. These studies have shown that exposure to acute psychological stressors (e.g., examinations and spousal discord) and chronic psychological stressors (e.g., nuclear disaster and caregiving) is associated with high antibody titers to these viruses. These viruses are thought to be controlled by normal host cell-mediated immune response. When stress reduces the cell-mediated immune response, the virus replicates and stimulates an antibody response that is typically nonprotective (Kiecolt-Glaser et al., 1991). In the case of genital herpes, Kemeny, Cohen, Zegans, and Conant (1989) showed that a negative mood state was correlated with a decrease in CD8+ lymphocytes (the principal effector against herpesvirus) and herpetic lesion recurrence. Likewise, psychological stress has been shown to predispose an individual to the onset of infectious mononucleosis (Glaser et al., 1991). This work remains

controversial, however, and the role of psychoneuroimmunology in latent viral infections, viral reactivation, and immune stimulation requires further investigation.

Fungal Infections

Although the association between emotional stress and infectious mycological disease has been long suspected, only recently has considerable attention been paid to this association. Fungal infections are well-known to be associated with the stressful conditions of pregnancy, surgical trauma, cancer, organ transplants, long-term antibiotic use, corticosteroid therapy, diabetes mellitus, critical illness, and prematurity (Reszel, Mishra, Mishra, & Pierson, 1993; Shareef, Myers, Nagabhushan, Mathews, & Witek-Janusek, 1998; Witek-Janusek, Cusack, & Mathews, 1998).

Stress hormones such as cortisol and adrenaline are known to enhance pathogenesis of experimental fungal disease (Odds, 1988). For example, *Candida albicans* and related fungi are endogenous opportunists, and infections with these fungi are typically associated with debilitating or predisposing conditions or both. *Candida* infections are the first symptom of active AIDS to appear in HIV-positive individuals. One factor shared by AIDS patients and the other susceptible individuals described previously is hormonal imbalance resulting from HPA axis activation. Furthermore, emotionally affected women who perceive their situation to be stressful have a higher incidence of vaginal *Candida* infections (Reszel et al., 1993). Candidiasis also appears frequently in people undergoing surgery, a unique form of stress that involves emotional stressors (anxiety), chemical stressors (anesthesia), and physical stressors (surgery) (Mishra et al., 1994). Similarly, the emotional stress of divorce has been positively correlated with increased incidence of the carriage of *Candida* (Reszel et al., 1993). Such associations of stress and fungal infection in vulnerable populations are only beginning to be understood.

➢ STRESS-IMMUNITY AND NURSING SCIENCE

The holistic view of human nature ascribed to by the discipline of nursing is harmonious with the philosophical underpinnings of psychoneuroimmunology (McCain & Smith, 1994; Zeller, McCain, & Swanson, 1996). As a result, nurse researchers have used a psychoneuroimmunological framework in their research and have made significant contributions to the scientific growth of this field. Nurse investigators have examined stress-immune interactions in a variety of immune-based illnesses, including asthma, HIV, and cancer. In addition, nurse scientists have documented the immunosuppressive nature of postoperative pain and the effects of stress on wound healing. A psychoneurouimmunologic framework has also been used to understand the immunologic implications of child birth and postpartal stress on maternal-infant well-being. Some of these studies are addressed in the following discussion.

Asthmatic symptoms can often be initiated and potentiated by stressful life events. Kang et al. studied the effect of examination stress in asthmatic and nonasthmatic adolescents. Their results revealed that examination stress produces significant alterations in circulating immune cell subsets and in both proliferative and cytolytic activities. No differences were found between the asthmatic and nonasthmatic adolescents, however. Both healthy and asthmatic adolescents reported similar levels of stress and similar changes in immune cell numbers and function (Kang, Coe, Karaszewski, & McCarthy, 1998; Kang, Coe, & McCarthy, 1996). The lack of a relationship between asthma status and social support was believed to be due to the stability and well-managed nature of this asthmatic population (Kang, Coe, McCarthy, & Ershler, 1997). In a similar study, examination stress in adolescent asthmatics produced a bias toward a Th2-like pattern of cytokine production compared to that of nonasthmatic adolescents (Kang, Coe, McCarthy, et al., 1997). These studies are suggestive and need to be

replicated in asthmatics with less stable disease and in naturalistic situations of more intense or chronic stress or both.

The laboratory of Gayle Page conducted a series of compelling experiments in rodents that showed that untreated postoperative pain led to impaired NK cell activity and enhanced tumor metastases (Ben-Eliyahu, Page, Yirmiya, & Shakhar, 1999; Page & Ben-Eliyahu, 1997; Page, Ben-Eliyahu, & Liebeskind, 1994; Page, Ben-Eliyahu, Yirmiya, & Liebeskind, 1993). In her model, rats were subjected to laparotomy and injected with NK cell-sensitive radiolabeled tumor cells that metastasized to the lung. Rats that were treated with morphine, and that exhibited signs of pain relief, had significantly less radiolabeled tumor in the lung, fewer metastatic lesions on the lung, and higher postoperative NK cell activity (Page et al., 1993, 1994). These results suggest that untreated postoperative pain leads to impaired immune function (e.g., reduced NK cell activity) and potentially increased organ localization of tumor emboli. In addition, this research group has demonstrated that the degree of postoperative pain immunosuppression is related to the estrus stage of the rat, suggesting that reproductive hormones may affect the stress-immune response to surgical stress (Ben-Eliyahu, Page, Shakhar, & Taylor, 1996). As a whole, the studies of Page and colleagues indicate that the treatment of pain is necessary not only to alleviate suffering but also to prevent pain-induced immunosuppression and possible tumor metastatic spread. Although these observations have been made in animal models, others have shown in humans that surgery for tumor resection leads to a postoperative reduction in NK cell activity compared to preoperative levels (Pollack, Lotzova, & Stanford, 1992). Evidence that healing and recovery from surgery are potentially altered by stress has also been provided by Wysocki (1996), who found that noise stress delayed wound healing in an animal model. Also, McCarthy, Ouimet, and Daun (1991) have provided evidence that noise stress alters lymphoid cell function needed for tissue repair. The previously discussed results support the supposition that an individual's psychological state can influence surgical recovery by altering various aspects of immunity (Kiecolt-Glaser, Page, Marucha, MacCullum, & Glaser, 1998).

Immunity and HIV Progression

Living with HIV is replete with multiple stressors, and nurse scientists have contributed to the supposition that the stress-endocrine-immune axis is implicated in HIV disease progression (McCain & Cella, 1995; McCain & Gramling, 1992; Robinson, Matthews, & Witek-Janusek, 1999a; Robinson et al., 1999b). Stress-induced neuroendocrine activation leads to elevations in plasma cortisol. *In vitro,* physiological concentrations of cortisol increase HIV replication in monocyte-derived macrophages, suggesting a potential role for stress hormones in HIV disease activation and progression (Swanson et al., 1998). The effectiveness of stress-reducing interventions in HIV disease has been evaluated by McCain et al. In a pretest-posttest design, the effect of a 6-week stress management program in HIV disease was evaluated (McCain et al., 1996). Outcome measures at 6 weeks and 6 months included perceived stress, quality of life, psychological distress, illness-related uncertainty, and CD4+ T lymphocyte levels. Although the program improved measures of emotional well-being, no significant changes in CD4+ lymphocyte levels were detected. It is likely that CD4+ lymphocyte number may not be a sensitive indicator of improvement in immune function, and other types of immunological assessment may yield more positive results.

In an ongoing intervention study, Robinson and colleagues are examining the efficacy of an 8-week, mindfulness meditation-based stress-reduction program on psychoimmune variables in HIV-positive individuals. These investigators are measuring NK cell activity, which is an important host defense mechanism against viral infections, and opportunistic microbial infections, which cause significant morbidity and mortality in HIV-positive

patients. Preliminary data from the study suggest a positive effect of this program on psychological well-being and immune status (Robinson et al., 1999a). Undoubtedly, nurse investigators will continue to explore the role of stress in HIV disease management and progression.

Stress-induced immunosuppression may have special relevance to the nursing care of cancer patients who undergo immunosuppressive therapies. The emotional stress of undergoing breast biopsy for cancer diagnosis has been clearly demonstrated and presents a useful human paradigm to study psychologic stress (Hooper, Mathews, & Witek-Janusek, 1997; Witek-Janusek & Mathews, 1999b). Anticipation of breast cancer diagnosis has been shown to alter immune cell subsets (Fillion, Lemyre, Mandeville, & Piche, 1996) and TH1 and TH2 cytokine production (Witek-Janusek & Mathews, 1999b). The effect of stress on gene transcription factors has been investigated in women undergoing diagnostic breast biopsy. Gene transcription factors play a significant role in the immune response and can regulate the production of cytokines (Wulczyn, Krappmann, & Scheidereit, 1996). In the diagnostic breast biopsy paradigm, nuclear localization in lymphocytes of two transcription factors, NF-kapB and AP-1, was decreased in women experiencing significant emotional stress, whereas when stress was relieved (post-biopsy) the nuclear localization of these gene transcription factors was similar to those of age-matched control women (Nagabhushan, Mathews, & Witek-Janusek, 2000). If and how these factors relate to the modification of immune function and to cancer outcome remain to be determined, but such studies will move this field to a molecular understanding of the effects of stress.

Nurse researchers have used a psychoneuroimmunologic approach toward understanding the impact of childbirth and postpartal stress on maternal child health. Maureen Groer and her research team at the University of Tennessee College of Nursing have demonstrated that childbirth stress leads to a reduction in maternal secretory immunoglobulin A (sIgA). This reduction was most pronounced in women who reported an increased state of anxiety. Women with very low or undetectable levels of sIgA had a greater incidence of postpartal complications, and their infants had more illnesses. These results indicate that the stress of childbirth can have profound effects on maternal immune function, which can alter the clinical course of mothers and that of their infants (Annie & Groer, 1991). Interestingly, Groer, Mozingo, et al. (1994) demonstrated that touch, provided by a 10-minute slow-stroke effleurage back rub, was shown to increase sIgA levels in elderly adults. It remains to be determined if such an intervention may blunt the decrease in sIgA observed during parturition. Other nurse investigators have demonstrated that glucocorticoid hormones can profoundly influence the pattern of cytokine production (i.e., colony-stimulating factors) from neonatal mononuclear cells obtained from umbilical cord blood. Such immunomodulation may alter the newborn's ability to resist infectious pathogens (Witek-Janusek & Mathews, 1999b).

The unique psychologic and immunologic relationship between a breast-feeding mother and her infant is an intriguing paradigm in which to evaluate the stress-immune relationship. Stress-induced alterations in maternal immunity in breast-feeding mothers could potentially alter their capability to provide optimal levels of immunoglobulins for their infants. Postpartal mothers of preterm infants report high levels of mood disturbance compared to the general population. Mothers who score higher on negative mood subscales of the Profile of Mood States produce less milk sIgA than those who report lower negative mood states. Conversely, mothers who report higher vigor and anger produce greater amounts of milk sIgA (Groer, Droppleman, & Mozingo, 1999). Interestingly, an inverse relationship between cortisol levels and sIgA in breast milk has been reported, such that the higher milk cortisol was associated with lower milk sIgA. It is plausible that increased maternal stress leads to elevated plasma and milk cortisol. Higher levels of milk cortisol may

impair milk immune cell production of immunoglobulins or other immune cell functions or both (Groer, Humenick, & Hill, 1994; Groer et al., 1999). Such stress-induced alterations in milk endocrine and immune composition may potentially impact the immunologic benefits that infants receive from breast milk and certainly require additional investigation. This is especially relevant to premature and low-birth-weight infants, who are at high risk for infectious illness.

➢ FUTURE DIRECTIONS AND NURSING IMPLICATIONS

The guiding premise of psychoneuroimmunology is that stress-induced impairment of immune function influences disease progression or response to therapy or both. These types of investigations are directed toward an understanding of the effect of the psychoendocrine stress response on the immune system, particularly within the context of cancer, autoimmune disease, infectious disease, and maternal child health. Nurses must recognize the potential effectiveness of biobehavioral approaches to the care of patients with immune-based disease. Such approaches to stress management may not only improve the quality of life and emotional well-being of targeted populations but also halt disease progression or complications from opportunistic infection or both.

Complementary and alternative therapies integrate preventive and curative therapies that consider the whole person and are used to "complement" traditional approaches to illness. The use of complementary and alternative therapies by American health care consumers has markedly increased; rigorous scientific testing of such practices has lagged behind, however (Eisenberg et al., 1998; Fontanarosa & Lundberg, 1998). Increased use of massage, touch, meditation, acupuncture, yoga, botanical herbs, guided imagery, and behavioral-based stress reduction programs has spurred a renewed interest in understanding the scientific basis for such approaches toward healing and health maintenance. This integrative biobehavioral, mind-body, therapeutic approach is harmonious with the view of psychoneuroimmunology and that of nursing science.

As discussed previously, the links among one's emotional state, neuroendocrine activity, and immune response are well described. Future emphasis needs to be placed on understanding the mechanism(s) of stress-induced immune dysregulation and the relationship between stress-induced immune dysregulation and health outcome. That is, do stress-induced changes in immunity alter health, and can stress-reducing interventions that strengthen immunity halt disease progression and improve health? These are critical questions that require intensive empirical investigations using human paradigms of stress. Such approaches will lead to a better understanding of disease and to better diagnosis, treatment, or both of stress-induced immune dysfunction. The results will provide the scientific foundation that will lead to the identification of individuals "at risk" for psychological distress and altered immune reactivity. Such identification will permit the development of psychologically based interventions designed to reduce stress, promote immunocompetence, and hence improve health. Such interventions may prove to be cost-effective additions to traditional forms of treatment or therapy or both and hold promise for disease control. Ultimately, this will serve to enhance the quality and the quantity of life.

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Aberrant Nuclear Expression of AP-1 and NFkB in Lymphocytes of Women Stressed by the Experience of Breast Biopsy

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We have investigated the expression of AP-1 and NFkB in peripheral blood lymphocytes of women scheduled for breast biopsy. Samples were collected when women were informed of the need for biopsy (prebiopsy, T1, 5–7 days prior to the actual biopsy) and 7–10 days after they learned the result of their biopsy (postbiopsy, T2). At the time of blood collection, psychological stress was evaluated using Speilberger's State Trait Anxiety Inventory (STAI) and the Profile of Mood States (POMS). Women scheduled to undergo breast biopsy reported significant increases in anxiety (STAI) and mood disturbance (POMS). Gel shift mobility assays showed that mitogen stimulated peripheral blood lymphocytes of these women were less capable of the nuclear expression of AP-1 or NFkB at T1. Similar assessments, 7–10 days after the women learned of the results of their breast biopsy, showed these same women to have a marked reduction in anxiety and mood disturbance and an increased nuclear translocation of AP-1 and NFkB. These results show a significant decrease in nuclear AP-1 and NFkB expression during the period of emotional distress prior to biopsy with a return of nuclear transcription activity to normal levels when distress was relieved. Several studies have correlated increased psychological stress with decreased immune function. The results of this study suggest that psychological stress may mediate immunosuppression by altering the expression of the transcription factors, AP-1 and NFkB.

Key Words: transcription factors; NFkB; AP-1; breast biopsy; psychological stress; human peripheral blood lymphocytes; gel shift assay; cancer.

INTRODUCTION

A number of studies have explored the relationship between psychological stress and the immune response (Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck, & Kiecolt-Glaser, 1987; Whiteside & Herberman, 1994). Those studies have assessed stress and immunity in medical students during classroom examinations, caregivers of diseased patients, individuals undergoing exercise-induced stress, volunteers exposed to experimental laboratory stress, and women with breast cancer (Glaser, Rice, Sheridan, Fertel, Stout, Speicher, Pinsky, Kotur, Post, Beck, & Kiecolt-Glaser, 1987; Whiteside & Herberman, 1994; Anderson, Farrar, Golden-Kreutz, Kreutz, Kutz, Maccallum, Courtney, & Glaser, 1998). Stressed individuals showed decreased lymphoblast transformation in response to mitogens (Dobbin, Harth, McCain, Martin, & Cousin, 1991); increased Epstein–Barr virus, Herpes simplex virus, and cytomegalovirus reactivation (Bonneau, Zimmerman, Ikeda, & Jones, 1998); dysregulation of cytokines (Marshall Jr, Agarwal, Loyod, Cohen, Henninger, & Morris, 1998); elevated secretion of proinflammatory cytokines; and decreased natural killer cell activity (Mae, Song, Lina, Jongh, Van Gastel, Kenis, Bosmans, De Meester, Benoy, Neels, Demedts, Janca, Scharpe, & Smith, 1998; Whiteside & Herberman, 1994).

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NFkB and AP-1 are transcription factors that regulate lymphocyte function. Nuclear expression of these transcription factors is increased during lymphocyte activation, cytokine secretion, and latent viral reactivation. Agents that are known to block lymphocyte activation are immunosuppressive and inhibit NFkB and AP-1 activity (Kopp & Ghosh, 1995). Thus we hypothesized that stress may alter the expression of these transcription factors. Experiments were designed to evaluate the expression of NFkB and AP-1 in the peripheral blood lymphocytes isolated from women pre- and postbreast biopsy. This experimental design provides a naturalistic psychological stress paradigm in which the effects of stress can be evaluated.

METHODS

Mononuclear cells were prepared from heparinized whole blood by Ficoll–Hypaque separation (Sigma, St Louis, MO). Mononuclear cells were cultured at a density of 1×10^6/ml/well in triplicate with or without phorbol 12-myristate 13-acetate and phytohemagglutinin (PMA + PHA), for 48 h at 37°C as described previously (Witek-Janusek & Mathews, 1999). Phenotypic analysis of mononuclear cells was performed with flurochrome conjugated antisera by flow cytometry with a Becton–Dickinson FACStar Plus System (Hialeah, FL). T lymphocytes were identified with anti-CD3ε, B lymphocytes with anti-Ig, and NK cells with anti-CD56, from PharMingen (San Diego, CA). The nonadherent cells (>99% lymphocytes as judged by Wright–Giemsa staining) were then harvested, treated with lysing buffer (10 mM Tris-HCl, pH 7.4, 2 mM magnesium chloride, 140 mM sodium chloride, 0.5 mM dithiothreotol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.1% tritonX100), and stored at −70°C. The samples were thawed at 37°C in a water bath. The nuclear extract was prepared as described previously (Singh & Aggarwal, 1995). NFkB and AP-1 double standard oligonucleotides (Promega, Madison, WI) were end-labeled with [γ-^{32}P]-ATP (Amersham, Arlington Heights, IL). One microliter (30,000 cpm) of labeled probe was incubated with 3 μl of nuclear extract (protein concentration, 5 μg) and binding buffer (15 mM Tris-HCl, pH 7.5, 7.5% glycerol, 75 mM sodium chloride, 1.5 mM EDTA, 1.5 mM dithiothreotol, 0.5 mM phenylmethylsulfonyl fluoride, 0.3% NP-40, and 20 μg bovine serum albumin), final volume 25 μl for 30 min at 25°C and separated with a 4% nondenaturing polyacrylamide gel for 1.5 h with 0.5% TBE (Singh & Aggarwal, 1995). The gels were vacuum dried for 1 h and exposed to X-ray film for 12 h at −70°C. The developed autoradiograms were densitometrically quantified with an Alphaimager 2000 version 4.03 (Innotech Corp, San Leandro, CA) video image analyzer. The specificity of shifted bands was confirmed by incubating first with a 10-fold excess of cold oligonucleotides and then with radioactively labeled oligonucleotides. Results are expressed as percentage of positive control (100%, PMA and PHA stimulated nuclear extracts from normal, nonstressed individuals) for each gel.

Sixteen women, 52.1 ± 3.7 years of age, were enrolled from the Loyola University Breast Care Center on the day that their physician recommended the need for a breast biopsy. After signing an informed consent, blood samples (16 ml) were obtained by venipuncture and the psychological assessments were administered in a private area adjacent to the clinic. At approximately 5–7 days after T1, the study women underwent breast biopsy. These same women were also sampled post biopsy (T2). T2 occurred 7–10 days after the women learned the results of their breast biopsy. At this time the women met with the study's nurse researcher at the Breast Care Center,

TABLE 1
Evaluation of Psychological Stress in Breast Biopsy Patients

Psychological test	T1, Prebiopsy	T2, Postbiopsy	Normal controls
POMS	22.0 ± 7.1[a]	0.2 ± 6.9	2.2 ± 9.3
STAI	42.7 ± 4.4[a]	27.8 ± 2.2	27.2 ± 1.7

Note. Values represent mean ± standard error of mean. POMS, Profile Of Mood State; STAI, State Trait Anxiety Inventory; T1, Time 1, prior to breast biopsy; T2, Time 2, 7–10 days after breast biopsy results were known.

[a] Statistically significant $p < .05$ from T2 and controls.

their blood was collected, and the psychological instruments were administered. Age-matched, nonstressed control women were solicited from within the University community. At a convenient time these women were met in a University office setting. In a procedure similar to that for the biopsy group, their blood was obtained by venipuncture and the same psychological instruments were administered. Control women were sampled at one time point only.

For the purposes of this study, anxiety was assessed using Speilberger's State Anxiety Inventory (STAI) and mood was assessed using the Profile of Mood States (POMS). The STAI measures the state of anxiety and it is designed to assess "state of mind" or anxiety at the moment. The STAI has alpha reliability coefficients of 0.83–0.92 and convergent validity with other anxiety instruments (alpha 0.75–0.80). The POMS is a reliable and valid tool designed to identify and assess general distress and mood. It has internal consistency reliabilities of 0.87–0.95, and stability coefficients (test–retest) of 0.65–0.74 (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1970; McNair, Lorr, & Droppleman, 1992). These instruments were used to reflect the level of psychological distress experienced by these women pre and post breast biopsy. This study was approved by the Institutional Review Board for the study of Human Subjects of Loyola University Medical Center.

RESULTS

Table 1 shows the psychological profile of women pre biopsy (T1) and post biopsy (T2). Mood disturbance and anxiety, as measured by the POMS and STAI, showed a significant ($p < .05$) decrease from pre- (T1) to postbiopsy (T2). These results demonstrated that the women experience two forms of psychological distress, anxiety and mood disturbance, at T1. At T2, reduced anxiety and improved mood were demonstrated and were not different from nonstressed, age-matched, control women. Electrophoretic mobility shift assays were used to quantify the AP-1 and NFkB activity in the stimulated peripheral blood lymphocytes of the subjects. Lymphocytes obtained at T1 exhibited lower expression of nuclear NFkB and AP-1 compared to T2 (Table 2). No change in nuclear localization of another transcription factor, Oct-1, was noted either before or after biopsy. Examples of the electrophoretic mobility shift assays are presented in Figs. 1, 2, and 3. The reduction in expression of AP-1 and NFkB was greater for NFkB than AP-1. Follow-up of these patients at the post-biopsy period (T2) showed NFkB and AP-1 activity to be higher ($p < .001$) than that observed at T1. From T1 to T2 the NFkB activity increased nearly threefold, while that of AP-1 nearly doubled. No phenotypic differences were observed in the women's lymphocyte populations from T1 to T2. The activity of the transcription

TABLE 2
Evaluation of Peripheral Blood Lymphocytes of Breast Biopsy Patients

Transcription factor	Percentage of Positive Control		Normal controls
	T1, Prebiopsy	T2, Postbiopsy	
NFkB	32.8 ± 5.3*	104.3 ± 3.2	105.9 ± 3.8
AP-1	57.4 ± 5.1*	107.8 ± 2.7	94.7 ± 5.1
Oct-1	101.8 ± 4.2	102.6 ± 3.9	100.9 ± 5.0
Lymphocyte Population %			
T	71.8 ± 9.0	72.2 ± 8.1	70.7 ± 5.6
B	16.1 ± 5.1	14.7 ± 4.8	17.8 ± 5.4
NK	13.0 ± 11.7	12.6 ± 9.2	11.3 ± 6.6

Note. Percentage of AP-1 and NFkB activity in the nuclear extracts of peripheral blood lymphocytes stimulated with PMA/PHA for 48 hrs. Decreased NFkB and AP-1 activity were observed pre-biopsy (T1). When the stress was relieved, post-biopsy (T2), AP-1 and NFkB nuclear activity increased. A positive normal (non-stressed) control (100% activity) was examined for each set of samples for calculating relative percentage activity. Values represent mean ± standard error of mean. No statistically significant differences were observed in the percentage of Oct-1 nuclear activity. No statistically significant differences were observed between the percentages of the individual lymphocyte populations analyzed between groups.

* Statistically significant $p < .001$ when compared to T2 and normal controls.

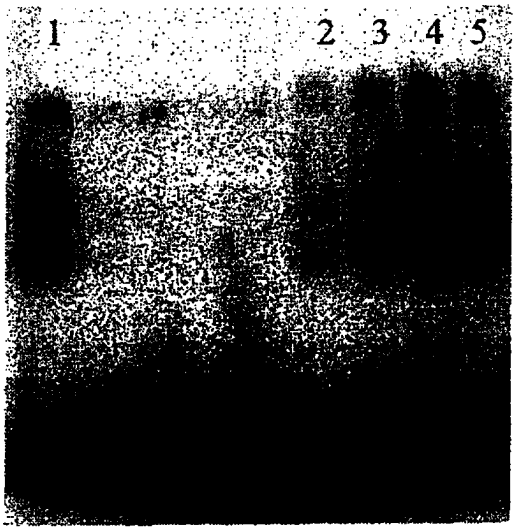

FIG. 1. Representative electrophoretic mobility shift assessment of NFkB nuclear localization in extracts from the lymphocytes of a woman pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lanes 2 and 3 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of a woman prior to biopsy. Lanes 4 and 5 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of the same woman post biopsy. Lanes 2–5 are extracts derived from the lymphocytes of one woman. Densitometric quantification of the autoradiographic results were calculated for both of the bands in each lane. The results for the higher molecular weight band are presented in Table 2.

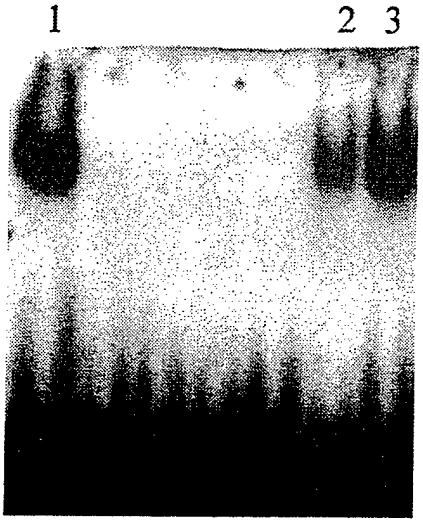

FIG. 2. Representative electrophoretic mobility shift assessment of AP-1 nuclear localization in extracts from the lymphocytes of a woman pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lane 2 contains the PMA and PHA stimulated nuclear extract from lymphocytes of a woman prior to biopsy. Lane 3 contains the PMA and PHA stimulated nuclear extract from lymphocytes of the same woman postbiopsy.

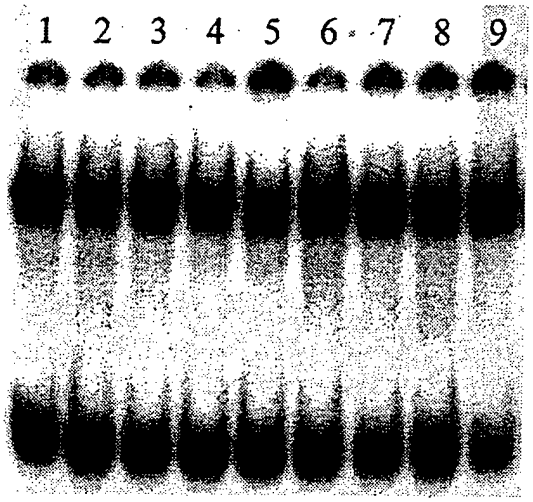

FIG. 3. Representative electrophoretic mobility shift assessment of Oct-1 nuclear localization in extracts from the lymphocytes of women pre- and postbiopsy. Lane 1 contains the PMA and PHA stimulated nuclear extract from the lymphocytes of a normal, nonstressed individual. Lanes 2, 3, 4, and 5 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of women prior to biopsy. Lanes 6, 7, 8, and 9 contain the PMA and PHA stimulated nuclear extracts from lymphocytes of women postbiopsy. Lanes 2–9 are extracts derived from the lymphocytes of four women and are presented sequentially with the following pairings (Lane 2) pre- and (Lane 6) postbiopsy from the same woman, (Lane 3) pre- and (Lane 7) postbiopsy from the same woman, etc.

factors in women after the experience of breast biopsy was similar to that of age-matched and nonstressed women.

DISCUSSION

We have examined nuclear localization of two transcription factors, NFkB and AP-1, in lymphocytes isolated from the peripheral blood of women scheduled to undergo biopsy for suspected breast cancer. The blood samples were collected at two different time intervals, before and after breast biopsy. The prebiopsy sampling occurred on the day the woman's physician informed her of the need for a breast biopsy. At this time the women exhibited increased anxiety and mood disturbance, which was most likely related to the uncertainty associated with an impending biopsy of the breast. From pre to postbiopsy there was a clear decrease in the anxiety and mood disturbance expressed by these women. These changes in anxiety and mood disturbance were reflected by changes in NFkB and AP-1. The nuclear levels of these transcription factors were decreased prebiopsy or during the time of heightened psychological stress; while at postbiopsy both the psychological stress and the transcription factors returned to that of nonbiopsied control women.

Experimental studies in animals and clinical studies in humans suggest that psychological stress decreases immune function and increases the risk for infectious disease and the reactivation of latent viruses. NFkB regulates the expression of gene products such as cytokines, acute phase proteins, and adhesion molecules. These are necessary components of immunity which ensure effective protection from infectious disease (Dobbin, Harth, McCain, Martin, & Cousin, 1991; Bonneau, Zimmerman, Ikeda, & Jones, 1998; Mae, Song, Lina, Jongh, Van Gastel, Kenis, Bosmans, De Meester, Benoy, Neels, Demedts, Janca, Scharpe, & Smith, 1998; Kopp & Ghosh, 1995). It is possible that a stress-induced decrease in NFkB and AP-1 activity may down regulate the secretion of important cytokines that promote an effective immune response and are required for optimal protection from infection. This is especially important for cancer patients undergoing immunosuppressive chemotherapy. Decreased immunity in cancer patients may not only increase susceptibility to infection but may also accelerate the growth and progression of cancer (Cohen & Rabin, 1998). In a recent study, reduced translocation of lymphocyte transcription factors was observed in women with breast cancer. Those authors speculated that a defect in transcription factor translocation may be related to breast cancer (Kurt, Urba, Smith, & Schoof, 1998). Although the design of this study does not permit a causal interpretation, the data does suggest that the emotional distress associated with impending breast biopsy may influence the capacity of lymphocytes to translocate nuclear transcription factors. Therefore, psychological stress may be an important modulator of transcription factors which, in turn, may result in reduced immune function at the level of transcription.

NFkB, AP-1, and other transcription factors act together to regulate and activate components of the immune system. To our knowledge, this is the first report suggesting a possible molecular mechanism whereby psychological stress may modulate immune function. Because NFkB, AP-1, and related transcription factors play a central role in the immune system (Kopp & Ghosh, 1995; Thomas, Tymms, McKinlay, Shannon, Seth, & Kola, 1997), they may be a marker or means by which to measure the overall effect of stress upon immune function.

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